PHYTOREMEDIATION OF HYDROCARBON-CONTAMINATED SOIL USING PLANTS ADAPTED TO THE WESTERN CANADIAN CLIMATE

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By

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ABSTRACT

As hydrocarbon contaminated sites occur throughout Canada, and are threats to ecosystem and human health, techniques to remediate them are needed. Phytoremediation is the use of plants and their associated microorganisms to degrade, sequester or contain contaminants in soil. This technique is gaining in popularity due to low cost and minimal soil disturbance. However, there are several barriers to implementation of phytoremediation biotechnology in Canada. Only a few species of plants that are native or non-native in Canada have been tested for hydrocarbon tolerance and/or degradation ability. Furthermore, the reason why some plants are more tolerant of hydrocarbons than others, and whether tolerant species also increase hydrocarbon degradation is still unknown. A series of experiments were executed to examine hydrocarbon tolerance in plants including botanical field surveys and growth chamber experiments.

Contaminated field plots had significantly higher soil pH, carbon to nitrogen ratio and bare ground, and lower total nitrogen, available phosphorus and litter cover. Mean diversity was 0.52 at the uncontaminated and 0.45 at the contaminated plots even though species richness was similar. Mean species similarity between contaminated and uncontaminated plots was only 31.1% and cover similarity 22.2%. The most common species observed on contaminated field soil were kochia (*Kochia scoparia* (L.) Schrad.), wild barley (*Hordeum jubatum* L.), salt grass (*Distichlis stricta* (Torr.) Rydb.), Canbyi bluegrass (*Poa canbyi* (Scribn.) Piper), western wheatgrass (*Agropyron smithii* Rydb.) and slender wheatgrass (*A. trachycaulum* var. *trachycaulum* (Link) Malte). Species that were non-native, non-mycorrhizal, annual, biennial, large seeded and that

reproduced by seed only were significantly more common on contaminated plots. Species that were non-mycorrhizal, self-pollinated, large seeded and that reproduced by seed only formed significantly more plant cover on contaminated plots, while woody species and those with unassisted or bird-dispersed seeds formed less.

The species with the highest survival after five weeks in a variety of crude oil-contaminated soils included one native and four non-native grasses, two native and three non-native legumes and two native forbs. All plants grown in potting soil contaminated with 5,000, 10,000 and 50,000 ppm crude oil had significantly lower total biomass and relative growth rates (RGR) compared to the control except Indian breadroot (*Psoralea esculenta* Pursh). As the crude oil concentration increased from 5,000 to 50,000 ppm total biomass became more strongly negatively correlated (from r=-0.674 to r=-0.939) with RGR.

In hydrocarbon-contaminated field soil, total biomass and RGR of eight species with seed masses covering four orders of magnitude were significantly lower than in uncontaminated soil. Both seed size (r=0.976) and RGR (r=-0.916) were strongly correlated with performance in hydrocarbon-contaminated field soil. Those species with large seeds and slow RGR were more tolerant than species with small seeds and high RGR.

The two most tolerant species were Indian breadroot and crested wheatgrass (*A. pectiniforme* R. & S.). After 16 weeks of growth crested wheatgrass was a better hydrocarbon degrader than Indian breadroot as the quantity of five unidentified hydrocarbons was significantly lower in the rhizosphere soil of the former species. When these two species were grown together in the same pot, individual crested

wheatgrass plants produced 16 mg more biomass on average than when grown in single species pots, suggesting that interplanting a legume and a grass benefits growth.

Hydrocarbon tolerance in plants was related to resource use. Plants possessing stress-tolerant traits performed better on hydrocarbon-contaminated soil than those species with competitive or ruderal traits. This is because the soil of contaminated sites had low fertility and/or adverse soil chemistry. The most tolerant species were not necessarily good at hydrocarbon degradation.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
C	Competitive
CCA	Canonical correspondence analysis
EC	Electrical conductivity (measurement of soluble salt concentration)
GC	Gas chromatograph
LSD	Least significant difference
R	Ruderal
RGR	Relative growth rate
PAH	Polycyclic aromatic hydrocarbon
S	Stress-tolerant
SLA	Specific leaf area
SPME	Solid-phase microextraction
VAM	Vesicular-arbuscular mycorrhiza

1.0 INTRODUCTION

The extraction of petroleum products to fuel our industrial society inevitably results in spills, due to human and mechanical error. Although there is no comprehensive list of contaminated sites in Canada, there are at least 5,000 sites that the federal government is responsible for remediating (McIntyre and Lewis, 1997). In Saskatchewan, there are at least several hundred hydrocarbon-contaminated sites (Germida et al., 2002). Contaminated sites pose a threat to human and ecosystem health, so measures must be taken to decontaminate them. Traditional engineering techniques to clean hydrocarbon-contaminated soils are often expensive, ranging from $20 to over $1,500 per ton of soil, and result in extensive disturbance of the site (Schnoor, 2002). The observation that some plants and microorganisms are capable of growing in hydrocarbon-contaminated soil prompted research into remediation using these organisms. Bioremediation uses only microorganisms for degradation and can be done *in situ* or *ex situ* (Schnoor, 2002). Costs for bioremediation range from $50 to $100 per ton (Schnoor, 2002).

Phytoremediation is the use of plants and their associated rhizosphere microorganisms to degrade, sequester or contain soil contaminants most commonly *in situ* (Cunningham et al., 1996). The cost of phytoremediation is relatively low ranging from $10 to $35 per ton (Schnoor, 2002). Preliminary research on phytoremediation reveals that it may be more effective than using microorganisms alone. Many

greenhouse, growth chamber and field studies note that growing plants in hydrocarbon-contaminated soil increases the rate of hydrocarbon degradation compared to unplanted controls (Aprill and Sims, 1990; Erickson et al., 1994; Schwab et al., 1995; Gunther et al., 1996; Reilley et al., 1996; Qiu et al., 1997; Pradhan et al., 1998; Siciliano and Germida, 1998a; Reynolds and Wolf, 1999). These promising results have prompted scientists to further investigate the potential of plant/microorganism combinations for remediation of contaminated soils.

Plants that are hydrocarbon tolerant have the least change in growth when comparing performance in contaminated to uncontaminated soil. Unfortunately, an explanation of the mechanisms conveying hydrocarbon tolerance in plants has not been adequately developed. Gudin and Syratt (1975) note that legumes are more common on hydrocarbon-contaminated field sites than plants in other families, and suggest that their ability to fix nitrogen is responsible. This is certainly possible because the main effect of hydrocarbons on plants, especially in situations where contaminated soils are weathered, is via alteration of soil fertility and water relations rather than direct toxic effects (Udo and Fayemi, 1975; McGill, 1977; Xu and Johnson, 1997). Evidence in support of this is the observation that fertilizer addition greatly improves plant growth in contaminated soil (Amadi et al., 1993; Cutright, 1995; Steffenson and Alexander, 1995; Lin and Mendelssohn, 1998).

Plants that are tolerant of hydrocarbons may be so because of their physiological traits. Species with large seeds and low relative growth rates (RGRs) perform better on infertile soil than those with small seeds and high RGRs (Grime and Hunt, 1975; Chapin, 1980). Ozone (Weinstein and Yanai, 1994) and heavy metal (Lasat, 2002)

tolerance is greater in plants with low RGRs. Whether physiological traits of plants affect hydrocarbon tolerance needs to be determined.

It is assumed that the most hydrocarbon tolerant plant species will also be the best hydrocarbon degraders. However, the relationship between hydrocarbon tolerance and degradation has not been extensively studied. In fact, there is some evidence that the most hydrocarbon tolerant species are not good at degrading hydrocarbons. There is no difference in hydrocarbon degradation between the most and the least tolerant plants when grown in weathered contaminated field soil (Kulakow et al., 2000). Corn (*Zea mays* L.), a plant with high root biomass in contaminated soil, degraded less phenanthrene than some plants with lower root biomass (Liste and Alexander, 1999). Plants likely improve microbial degradation of hydrocarbons by producing chemicals like phenols (Hedge and Fletcher, 1996; Liste and Alexander, 1999). A plant may be hydrocarbon tolerant but not produce chemicals that aid microbial degradation. Thus it is possible that some of the most tolerant plant species will be less successful at hydrocarbon degradation than less tolerant species.

One problem preventing the implementation of phytoremediation technology in Canada is that few hydrocarbon tolerant plants are suitable for use in our country. To date, most research has focused on annual crop plants, perennials that are cold intolerant and species non-native to Canada (Germida et al., 2002). While crop plants may prove useful in the reclamation of cropland, they are not suitable for use in native pastures or areas where minimal soil disturbance is desirable. Cold-intolerant perennials have limited usefulness, as they would not be able to survive Western Canada's harsh winters. Importing non-native plants into the country is generally not allowed by

Canada Customs because of concerns that they will become noxious weeds. Furthermore, there are government restrictions regarding which species can be used for reclamation of native ecosystems (Jorgenson, 1997).

The purpose of this research was to determine which species were most tolerant of hydrocarbons in soil, and thus potential phytoremediators. The overall hypothesis was that the physiological traits of a plant would determine its hydrocarbon tolerance.

My research project had two goals:

1) Identify plants that tolerate hydrocarbons in soil.
2) Determine if the two most hydrocarbon tolerant plant species are able to degrade hydrocarbons.

This was accomplished through a series of studies designed to:

1) Document plant communities that naturally colonize hydrocarbon-contaminated field plots and compare them to communities on uncontaminated plots (Chapter 3).
2) Identify hydrocarbon tolerant plant species by comparing survival, biomass production and RGR in hydrocarbon-contaminated and uncontaminated soil (Chapter 4).
3) Determine if seed size and RGR are linked to hydrocarbon tolerance (Chapter 5).
4) Determine if the two most tolerant plant species degrade hydrocarbons in contaminated field soil (Chapter 6).

2.0 LITERATURE REVIEW

2.1 Mechanisms for Phytoremediation of Hydrocarbons

Phytoremediation is the use of plants and their associated microorganisms to remove, sequester or degrade contaminants in soil (Cunningham et al., 1996). Phytoremediation is a proposed technique to remediate both inorganic contaminants like heavy metals (Brown et al., 1994; Pilon-Smits et al., 1999), and organic contaminants like pesticides (Siciliano and Germida, 1998b) and petroleum hydrocarbons (Aprill and Sims, 1990). Plants useful for remediation of inorganics are species that hyper-accumulate the element of concern (Banuelos et al., 1997). The plants are eventually harvested, removing the metals from the soil; this is called phytoextraction (Ebbs et al., 1997). Some plants can also volatilize (i.e. transfer the contaminant to the atmosphere) heavy metals (Zieve and Peterson, 1984, Pilon-Smits and Pilon, 2002).

There are three mechanisms by which organic contaminant phytoremediation can occur: degradation, stabilization, and volatilization (Sims and Overcash, 1983; Cunningham et al., 1996; Siciliano and Germida, 1998b). While stabilization or volatilization is acceptable in some situations, degradation of the contaminant into non-toxic compounds is the most desirable outcome.

2.1.1 Degradation

Degradation occurs when hydrocarbons are broken down into simpler and usually less toxic compounds (Eweis et al., 1998). Plants and microorganisms in isolation, and in association, degrade hydrocarbons. However, the ability to degrade hydrocarbons is much less common in plants than in microorganisms.

There are only a few papers that document phytodegradation of hydrocarbons. Soybean (*Glycine max* (L.) Merr.) degrades [^{14}C]anthracene (Edwards et al., 1982) and bush bean (*Phaseolus vulgaris* L.) degrades [^{14}C]anthracene and [^{14}C]benz[*a*]anthracene (Edwards, 1988) when grown in solution culture. Durmishidze (1977) notes that corn and poplar (*Populus* spp. L.) degrade methane, and that corn also degrades benzene, toluene and xylene. Radiolabelled methane, ethane, propane, butane and pentane are assimilated by corn, vetch (*Vicia* spp. L.), grape (*Vitis vinifera* L.), walnut (*Juglans* spp. L.) and quince (*Cydonia oblonga* P. Mill.) (Durmishidze, 1977). Enzymatic oxidation, reduction and hydrolysis reactions in plants reduce substrate toxicity (Walton et al., 1994). Durmishidze (1977) summarizes the general conversion path that plants use to degrade *n*-alkane as:

n-alkane → primary alcohols → fatty acids → acetyl-CoA → various compounds

However, since the sterility of these systems was not discussed, it is possible that microorganisms were involved in the degradation process. Microorganisms may have been present on the seeds or in the soil and could have been responsible for some of the degradation that occurred.

Rhizodegradation of hydrocarbons occurs both aerobically and anaerobically, although the former is more rapid (Itävaara et al., 2000). Microorganisms like bacteria (Bossert and Bartha, 1984; Haigler et al., 1988; Boldrin et al., 1993; Brown et al., 1998), actinomycetes (Radwan et al., 1995) and fungi (Raymond et al., 1976; Sutherland, 1992; Donnelly and Fletcher, 1994) degrade hydrocarbons aerobically.

The first step in aerobic degradation of hydrocarbons is uptake by the microorganism (Gottschalk, 1986). The more water-soluble a compound is, the more readily it is taken up (Aprill and Sims, 1990). This is because most microorganisms require that a compound be in aqueous phase before they can metabolize it, although there are some exceptions (White and Alexander, 1996). Microorganisms may excrete biosurfactants that emulsify hydrocarbons mediating their transfer to the cytoplasmic membrane (Itävaara et al., 2000).

The second step in degradation of both aliphatic and aromatic hydrocarbons is incorporation of oxygen. Aliphatic hydrocarbon degradation by bacteria is catalyzed by monooxygenase according to the following formula (Gottschalk, 1986):

$$\text{Substrate-H} + O_2 + AH_2 \xrightarrow{\text{monooxygenase}} \text{substrate-OH} + A + H_2O$$

The resulting primary alcohol is oxidized to form a fatty acid. Aromatic hydrocarbon degradation by bacteria involves the incorporation of two oxygen molecules, a reaction that is catalyzed by dioxygenase (Sims and Overcash, 1983) (Figure 2.1A).

The third step in aromatic degradation is ring fission either between the two hydroxylated carbon atoms or adjacent to the hydroxyl groups (Volkering, 1996). The end products of these reactions are phenols and carboxylic acids, which are then used in the Krebs cycle (Sims and Overcash, 1983). There are only a few bacteria that grow on

ethane, propane, butane and hydrocarbons up to C_8 (Gottschalk, 1986). The degradation pathways of many 2- and 3-ring polycyclic aromatic hydrocarbons (PAHs) like naphthalene (Walton et al., 1994) are known, but the pathway for most 4- to 5-ring hydrocarbons has not yet been determined.

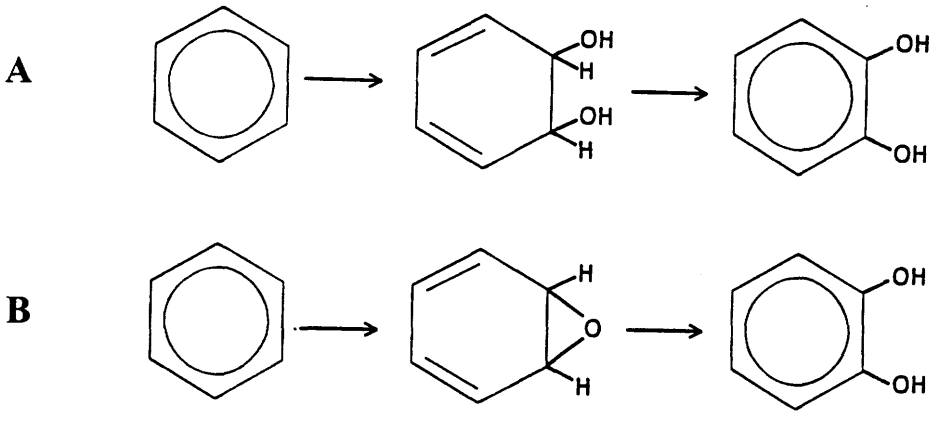

Figure 2.1. Initial steps in the microbial catabolism of aromatic hydrocarbons A: bacteria; B: fungi. From Volkering, 1996.

Fungi use a slightly different aerobic process than bacteria. Monooxygenase catalyzes the reaction of both aliphatic and aromatic hydrocarbons (Sutherland, 1992). For example, naphthalene degradation by fungi involves epoxidation to form an unstable arene oxide that is immediately either rearranged nonenzymatically to form phenols or hydrated to *trans*-dihydrodiols by epoxide hydrolase (Sutherland, 1990) (Figure 2.1B). Although most metabolites of fungal hydrocarbon degradation are non-toxic, small quantities of mutagenic and carcinogenic compounds may form depending on the species and type of hydrocarbon (Sutherland, 1992).

Anaerobic decomposition is performed mainly by bacteria that use anaerobic respiration or interactive fermentation/methanogenic metabolism (Riser-Roberts, 1998). A variety of organisms can anaerobically degrade hydrocarbons, including denitrifying bacteria, sulfate-reducing bacteria and methanogens (Riser-Roberts, 1998). However, usually more than one species of bacteria is required for complete anaerobic degradation of a hydrocarbon (Riser-Roberts, 1998). During anaerobic degradation of hydrocarbons the substrate is partially reduced and partially oxidized to form carbon dioxide and methane (Leahy and Colwell, 1990). Rather than using O_2 as an electron acceptor, an alternative, such as NO_3^-, SO_4^{-2} or CO_2 is used (Riser-Roberts, 1998). The general reaction scheme for anaerobic degradation is (Riser-Roberts, 1998):

Substrate + (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{-2}, CO_2) → Biomass + CO_2 + (N_2, Mn^{2+}, Fe_2, S_2, N_2O, CH_4)

Compounds that are resistant to anaerobic degradation include anthracene, naphthalene, benzene, aniline, 4-toluidine, 1- and 2-naphthol, pyridine and saturated alkanes (Riser-Roberts, 1998). Other compounds (e.g. low molecular weight halogenated hydrocarbons) can be degraded under anaerobic conditions (Riser-Roberts, 1998). Some compounds are degraded by one kind of anaerobic bacteria but not another. For example, straight-chain and branched alkanes and alkenes are not degraded under methanogenic conditions (Riser-Roberts, 1998). Anaerobic degradation is likely less important than aerobic degradation when plants are present as they tend to aerate the soil (Hutchinson et al., 2001b).

It is well known that in the rhizosphere (i.e. the zone of soil affected by plant roots) bacterial populations are 5 to 100 times higher than in bulk soil (Paul and Clark,

1989; Anderson et al., 1993; Atlas and Bartha, 1998) (Table 2.1). This is due to the release of root exudates like sugars, alcohols, acids, oxygen, sloughed cells and mucigel that provide carbon and energy for microorganisms (Schnoor et al., 1995; Cunningham et al., 1996). The presence of plants is believed to increase hydrocarbon degradation by stimulating the microbial community via the rhizosphere effect. Several studies support this hypothesis. Degradation of PAHs is usually higher in planted soils compared to unplanted in both greenhouse (Aprill and Sims, 1990; Reilley et al., 1996; Hutchinson et al., 2001b) and field studies (Qiu et al., 1997). Greater degradation is thought to occur due to higher numbers of hydrocarbon degraders in the rhizosphere. Benzene-, toluene- and xylene-degrading microorganisms (BTX) are five time more abundant in poplar tree rhizosphere soil than bulk agricultural soil (Jordahl et al., 1997). This means that microorganisms capable of degrading BTX compounds are present in uncontaminated soil but more abundant in rhizosphere soil.

Plants stimulate hydrocarbon-degrading bacteria by releasing root exudates (Donnelly et al., 1994; Hegde and Fletcher, 1996; Miya and Firestone, 2001). Some plant-released cometabolites (i.e. chemicals required for microbial degradation of other chemicals) aid in the degradation of hydrocarbons (Ferro et al., 1997). Plant enzymes like laccase, nitrilase, nitroreductase, peroxidase and dehalogenase are causative agents in the transformation of soil contaminants, although some of these enzymes cannot co-occur (Schnoor et al., 1995). Why plants release these chemicals is not known. Siciliano and Germida (1998b) note that there are likely both specific and nonspecific interactions between plants and microorganisms. Specific interactions occur when the plant produces a signal in response to a contaminant (Walton et al., 1994). Non-specific

interactions arise from normal plant processes that stimulate microorganisms (Siciliano and Germida, 1998b). Whether an interaction is specific or non-specific can be difficult to ascertain.

Table 2.1. Numbers of bacteria at increasing distance from the root surface.

Distance to Surface (mm)	Estimated Frequency (10^9 cells cm^{-3})	Distinguishable Morphological Types
0-1	120	11
1-5	96	12
5-10	41	5
10-15	34	2
15-20	13	2

From Paul and Clark, 1989.

Plants also stimulate microbial degradation by improving the physical and chemical properties of soil (Cunningham et al., 1996). Plants transport oxygen to the rhizosphere, increasing populations of aerobic microorganisms; this is particularly important if the soil is saturated with water (Cunningham et al., 1996; Hutchinson et al., 2001b). Plants contribute organic matter to soil in the form of exudates and sloughed cells while they are alive and as litter when they die (Miya and Firestone, 2001). The addition of organic matter increases soil fertility, improving microbial growth (Paul and Clark, 1996). By bringing together dense populations of microorganisms, plant roots may facilitate the exchange of genetic material that confers hydrocarbon tolerance and

degradation ability (Cunningham et al., 1996). Plant roots may also provide substrates for cometabolism (Cunningham et al., 1996).

The results of a few experiments suggest that degradation is not enhanced by the rhizosphere effect (Ferro et al., 1994, 1997; Kulakow et al., 2000). This is certainly possible since the strength of the rhizosphere effect varies from species to species. For example, the non-native crested wheatgrass (*Agropyron pectiniforme* R & S.) releases fewer root exudates than the native grasses western wheatgrass (*A. smithii* Rydb.) and blue grama (*Bouteloua gracilis* (HBK.) Lag.), which in turn affects microbial populations (Biondini et al., 1988). In weathered contaminated soil, PAHs may not be bioavailable (Kulakow et al., 2000), decreasing microbial degradation. Furthermore, in contaminated soils where growth conditions for plants are not optimal, a longer period of time may be needed to demonstrate significant degradation. Competition between plants and microorganisms for nutrients may also affect hydrocarbon degradation (Hutchinson et al., 2001a). Vavrek and associates (2002) found that hydrocarbon degradation is highest when only fungi and bacteria are added to the soil; the lowest amount of degradation occurs when plants and fungi, and plants and bacteria are grown together. This is because hydrocarbon addition to soil increases the C:N ratio. Microorganisms in the soil use the carbon for their energy source, immobilizing nitrogen in the process and making it less available to plants (Xu and Johnson, 1997). For degradation to occur the addition of nutrients, particularly nitrogen and phosphorus, may be required to reduce competition between plants and microorganisms (Hutchinson et al., 2001a).

2.1.2 Stabilization

Stabilization occurs when a plant reduces the bioavailability of a contaminant; the contaminant is not, however, degraded. This method of phytoremediation is useful in situations where prevention of ground water contamination is desired, or where the contaminant is not mobile or toxic to humans (Cunningham et al., 1996).

One stabilization method occurs when hydrocarbons accumulate in plant tissues (Durmishidze, 1977). Hydrocarbons accumulate in the roots of carrot (*Daucus carota* L.) (Wild and Jones, 1992), leaves of duckweed (*Lemna gibba* L.) (Duxbury et al., 1997), roots and leaves of soybean (Edwards et al., 1982) and stems and leaves of bush bean (Edwards, 1988). However, not all plants accumulate hydrocarbons. No plant species tested for their ability to accumulate oily sludge (Biederbeck et al., 1993), PAHs (Reilley et al., 1996; Qui et al., 1997), fuel oil (Chaîneau et al., 1997), a mixture of organic chemicals (Rogers et al., 1996), [^{14}C] anthracene or [^{14}C] benzo[a]pyrene were able to do so (Goodin and Webber, 1995). Species with a high lipid content accumulate more hydrocarbons than those with a lower lipid content (Schwab et al., 1998).

Chemical trapping by plant roots is another stabilization mechanism, particularly in areas where evaporation exceeds precipitation (Davis et al., 1994). Plant roots sometimes prevent hydrocarbons dissolved in water from moving through soil due to the upward water pumping action. Leaching of PAHs is significantly lower in pots planted with a mixture of eight prairie grasses than in unplanted pots (Aprill and Sims, 1990). A negligible amount of PAHs are found in leachate collected from pots planted with alfalfa (*Medicago sativa* L.), tall fescue (*Festuca arundinaceae* Schreber), sudangrass (*Sorghum vulgare* Pers.) and switchgrass (*Panicum virgatum* L.) (Reilley et

al., 1996). However, when tall fescue is grown in aged petroleum-contaminated soil there is no difference in the amount of hydrocarbons in the leachate between unplanted and planted pots (Hutchinson et al., 2001a). This may be because aged contaminated soils contain few water-soluble compounds.

2.1.3 Volatilization

In some cases chemicals are neither degraded nor stabilized; they are volatilized into the atmosphere. Plants absorb some chemicals through their roots and then release them into the atmosphere through stomata (Wiltse et al., 1998). Naphthalene volatilization is enhanced by the presence of bell rhodesgrass (*Chloris gayana* Kunth) (Watkins et al., 1994). Nitrobenzene volatilization is a major route of chemical loss when soybean, barley (*Hordeum vulgare* L.), hybrid poplar (*Populus* x *robusta* Schneid.) and honeysuckle (*Lonicera tatarica* L.) are grown in soil contaminated with this chemical (McFarlane et al., 1990). About 10% of diesel range hydrocarbons (C^{11}-C^{16}) in soil are volatilized over 150 days when planted with grasses (Pichtel and Liskanen, 2001). Diesel volatilization over 360 days is 58% when white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) are grown together (Kroening et al., 2001). Larger hydrocarbons may be less likely to volatilize: less than 2% of benzo(a)pyrene (a five-ring hydrocarbon) loss from silty loam soil planted with tall fescue is due to volatilization (Banks et al., 1999). Concerns regarding volatilization arise when the chemical is potentially dangerous in its gaseous form.

2.2 Factors Affecting Phytoremediation

2.2.1 Environmental factors

Environmental factors that affect the success of phytoremediation include soil texture, organic matter content, pH, oxygen availability, moisture, fertility, temperature, solar radiation and weathering. These factors affect the properties and bioavailability of hydrocarbons, germination and productivity of plants, and colonization and growth of rhizosphere microorganisms.

Sand does not bind molecules as readily as silt or clay, so the bioavailability of hydrocarbons is higher in sandy soils (Edwards et al., 1982; Charmicheal and Pfaender, 1997). The higher hydrocarbon bioavailability and hydraulic conductivity of sand means that spills on sandy soils are more likely to result in ground water contamination than spills on heavier textured soils. Organic matter and clay tend to bind lipophilic compounds, decreasing bioavailability of this material to plants, although not necessarily to soil microorganisms (Leahy and Colwell, 1990; Otten et al., 1997). The addition of high carbon organic matter like sawdust improves plant germination by decreasing hydrocarbon bioavailability to plants, but decreases yield due to an increase in the C:N ratio (Amadi et al., 1993). Plants require different soil textures and organic matter contents for optimal germination and growth (Blake, 1935; Evans et al., 1977). When screening plants for phytoremediation those species naturally adapted to the soil texture at the contaminated site will likely be more successful than those adapted to different soil textures. Clay and organic matter content also affects microbial populations via their ability to form soil aggregates (Paul and Clark, 1996).

Most vascular plants cannot tolerate soil with a very high or low pH, performing optimally between pH 5 and 8 (Barbour et al., 1987). Below pH 3 and above pH 9 root cell protoplasm is damaged (Larcher, 1980). However, the main effect of pH on plants is via alteration of nutrient availability. The availability of nitrogen, phosphorus, potassium, sulfur, calcium and magnesium decreases steadily as pH drops below 6 (Larcher, 1980). As well, aluminum and manganese reach toxic levels below pH 6.5 (Barbour et al., 1987). In alkaline soils (i.e. pH>8) iron, manganese and phosphate ions become fixed in relatively insoluble compounds and are thus less available (Larcher, 1980). Most bacteria operate optimally at a near neutral pH while fungi tolerate more acidic conditions (Leahy and Colwell, 1990). Very low or high pHs inhibit bacterial and fungal activity, decreasing the rate at which hydrocarbons are degraded (Lewis et al., 1984). Biodegradation of hydrocarbons doubles when soil pH increases to 7.4 from 4.5 (Verstraete et al., 1976).

Soil contaminated with hydrocarbons may have low oxygen availability (Cunningham et al., 1996). Lack of oxygen reinforces seed dormancy of some plants, preventing germination in contaminated soil (Baker, 1970; Amakiri and Onofeghara, 1984; Amadi et al., 1993). As the most effective hydrocarbon degrading microorganisms are aerobic, lack of oxygen can negatively affect this process (Atlas, 1981; Eweis et al., 1998). Extensive tillage improves soil aeration, which encourages the growth of aerobic hydrocarbon-degrading bacteria (Bollag et al., 1994; Genouw et al., 1994; Loehr and Webster, 1996).

Moisture is the factor most often limiting in the Prairie ecozone, especially in the most southern parts. Plant germination requires adequate quantities of water (Blake,

1935). Exposure to drought can cause the death of young seedlings (Blake, 1935), and decrease hydrocarbon degradation by bacteria (Holman and Tsang, 1995). Too much moisture impedes degradation, as anoxic zones in the soil suppress aerobic bacterial growth (Eweis et al., 1998) and hydrocarbon degradation (Holman and Tsang, 1995), and suffocate plant roots (Barbour et al., 1987). A soil water content of 60% is the ideal amount for degradation of hydrocarbons in loamy soil (Hutchinson et al., 2001b).

Low fertility can inhibit plant and microbial growth (particularly if a species is adapted to highly fertile sites), which in turn negatively affects phytoremediation (Abujnah, 1999). The addition of fertilizers (Amadi et al., 1993; Cutright, 1995; Steffenson and Alexander, 1995; Lin and Mendelssohn, 1998), surfactants (Madsen and Kristensen, 1997) and animal (Amadi et al., 1993) and green manures (Biederback et al., 1996) improves hydrocarbon degradation rates. Animal manure increases plant yield more than inorganic fertilizers, likely due to the binding of hydrocarbons to organic matter (Amadi et al., 1993). Nitrogen and phosphorus are more limiting in freshly contaminated than in aged contaminated soil as they tend to be immobilized by microorganisms shortly after contamination and mineralized when the C:N ratio decreases (Hutchinson et al., 2001a).

Temperature affects the availability and toxicity of oil, and plant and microorganism growth. Heat decreases the viscosity of hydrocarbons making them more bioavailable (Atlas, 1981), less soluble in water (Leahy and Colwell, 1990) and phytotoxic (Baker, 1970). The optimal temperature for dry matter production is between 5° and 40°C (Larcher, 1980). Indirectly, high temperatures lead to water stress, which decreases plant productivity (Larcher, 1980). Microorganisms benefit from heat;

hydrocarbon degradation rates double for every 10°C increase in temperature (Dibble and Bartha, 1979; Wright et al., 1997; Eweis et al., 1998).

Solar radiation modifies PAHs by increasing their polarity, water solubility and toxicity while they are in the soil (Huang et al., 1993; Ren et al., 1994; McConkey et al., 1997) and in plant tissue (Duxbury et al., 1997). The toxic effect of PAHs on plant tissue increases with exposure to ultraviolet radiation (Duxbury et al., 1997).

Weathered contaminated field soil contains fewer easily degradable compounds and more recalcitrant ones (Riser-Robert, 1998). The recalcitrant compounds are less bioavailable, as they adhere to clay and organic matter (Bossert and Bartha, 1984; Cunningham et al., 1996). While plants grown on soils with lower hydrocarbon bioavailability may exhibit better growth than soils with higher bioavailability, the goal of decontamination will not be met if the compounds are not degraded.

2.2.2 Biological Factors

Biological factors that may affect phytoremediation include degradation ability of associated microorganisms, and plant root architecture, growth rate, exudate production and productivity.

Research on hydrocarbon degrading microorganisms shows that some are more effective than others (Lewis et al., 1984; Madsen and Kristensen, 1997). The most important hydrocarbon-degrading bacteria and fungi are listed in Table 2.2. Uncontaminated soils generally have lower numbers of hydrocarbon degrading species than soils that have been contaminated, because the microbial community adapts to the presence of hydrocarbons (Leahy and Colwell, 1990). Adaptation occurs via (i)

induction and/or depression of enzymes, (ii) genetic changes resulting in new metabolic abilities, and (iii) selective enrichment of organisms (Leahy and Colwell, 1990).

Table 2.2. Soil microorganisms that degrade hydrocarbons.

Bacteria		Fungi	
Achromobacter	*Mycobacterium*	*Acremonium*	*Mortierella*
Acinetobacter	*Nocardia*	*Aspergillus*	*Paecilomyces*
Alcaligenes	*Proteus*	*Aureobasidium*	*Penicillium*
Arthrobacter	*Pseudomonas*	*Beauveria*	*Phoma*
Bacillus	*Rhodococcus*	*Botrytis*	*Phanerochaete*
Brevibacterium	*Sarcina*	*Candida*	*Pleurotas*
Chromobacterium	*Serratia*	*Chrysosporium*	*Polyporus*
Corynebacterium	*Spirillum*	*Cladosporium*	*Rhodotorula*
Cytophaga	*Streptomyces*	*Cochliobolus*	*Saccharamyces*
Erwinia	*Vibrio*	*Cunninghamella*	*Scolecobasidium*
Flavobacterium	*Xanthomonas*	*Cylindrocarpon*	*Sporobolomyces*
Micrococcus		*Debaryomyces*	*Sprotrichum*
		Fusarium	*Spicaria*
		Geotrichum	*Syncephalastrum*
		Gliocladium	*Tolypocladium*
		Graphium	*Torulopsis*
		Humicola	*Trichoderma*
		Monilia	*Verticillum*

From Germida et al., 2002 and April et al., 2000

Plants with extensive fibrous root systems, like grasses, are considered the most effective phytoremediators, as they explore larger volumes of soil than plants with taproots (Aprill and Sims, 1990). Plants with herringbone root morphology are more effective at soil exploration than plants with random or dichotomous morphologies (Fitter et al., 1988). Studies on root architecture in mixed prairie show that while grasses form dense mats of roots in the top 0.5- to 1-m of soil, many tap rooted species typically reach soil depths greater than one metre, some up to four metres (Albertson, 1937). Thus while grasses may be valuable for phytoremediation of soils with shallow contamination, certain taprooted forbs may be more effective for remediation of deeper contamination.

Slow growing plants may have higher specific root lengths and relatively more fine roots than faster growing plants (Boot and Mensink, 1990). Furthermore, when faced with nutrient shortages, slow growing species produce longer root hairs than fast growing species (Boot and Mensink, 1990). Slow growing species from infertile habitats may release more root exudates than fast growing species to facilitate the acquisition of nutrients that would otherwise be unavailable (Lambers and Poorter, 1992). Since root exudates are hypothesized to improve degradation (Cunningham et al., 1996), using species that produce more exudates may be advantageous.

Plants with high productivity have more root biomass and probably higher populations of rhizosphere microorganisms. Plants that are able to sustain their growth in contaminated soil would be more successful at phytoremediation than plants that cannot. However, plant productivity in uncontaminated soil is not indicative of productivity in hydrocarbon-contaminated soil (Kulakow et al., 2000). The plant

species with the highest productivity in uncontaminated soil, barley, has the lowest productivity in hydrocarbon-contaminated soil (Kulakow et al., 2000). Plant productivity is limited in hydrocarbon-contaminated soil largely due to low available nitrogen (Biederback et al., 1993). Improved grass productivity when interplanted with legumes occurs even over short lengths of time (Ta and Faris, 1987), suggesting that legumes release nitrogen in their root exudates (Wacquant et al., 1989). Thus interplanting hydrocarbon-tolerant legumes with grasses may result in greater plant community productivity than with a monoculture.

2.3 Effects of Hydrocarbons and Industrial Disturbance on Soil

Hydrocarbon spills may or may not be associated with industrial disturbance. Oceanic oil spills that wash up on shore and large pipeline spills may affect land that has not been impacted by humans. In these cases, only the presence of hydrocarbons affects soil properties. However, many terrestrial oil spills are associated with industrial disturbance. Oil spills often occur during the drilling process on land altered to improve equipment and vehicular access, or along pipelines where soil was disturbed during the installation process. Hydrocarbon contamination may also occur at industrial sites in urban areas. In these situations, both hydrocarbon contamination and disturbance of land will affect soil properties. Most of the time soil disturbance and hydrocarbon contamination affect soil properties in a similar way. When soil disturbance and hydrocarbon contamination have the opposite effect on soil properties, they may cancel each other out.

2.3.1 Physical soil properties

Hydrocarbons affect aggregation, soil water holding capacity, bulk density and temperature. With severe hydrocarbon contamination, soil aggregates break down, causing dispersion (Ellis and Adams, 1961). Water holding capacity decreases dramatically with the addition of hydrocarbons. No water is able to infiltrate soil contaminated with 150-mL crude oil kg^{-1} for 100 minutes (Khalimov et al., 1996). Water holding capacity slowly increases as microorganisms degrade hydrocarbons (Toogood, 1977). Bulk density decreases in soil saturated with natural gas, due to the increase in organic matter (Adams and Ellis, 1960). The addition of hydrocarbons increases soil temperature as it darkens the soil colour, decreasing reflection and increasing light absorption (Biederbeck et al., 1993). Haag and Bliss (1974) note that application of crude oil to Arctic tundra decreases soil reflectivity by 50%, which in turn increases soil temperature.

Industrial disturbance can affect soil texture and bulk density. Pipeline construction increases the quantity of clay by mixing the A horizon with the B and C horizons (Naeth et al., 1987). Both pipeline (Naeth et al., 1987), wellsite (Hammermeister, 2001) and road (Dick et al., 1988) construction significantly increases bulk density. This increase is due to soil horizon mixing and compaction from vehicular traffic (Naeth et al., 1987).

2.3.2 Chemical soil properties

The addition of petroleum hydrocarbons to soil affects pH, electrical conductivity, redox potential and fertility. In some studies hydrocarbon addition

increases pH (Udo and Fayemi, 1975; Biederbeck et al., 1993) while in others the opposite occurs (Amadi et al., 1994) possibly due to differences in when the soil sample was tested. Hydrocarbon bioremediation may temporarily decrease pH due to organic acid accumulation (Eweis et al., 1998). Ellis and Adams (1961) conclude that soil pH is buffered by hydrocarbons to a neutral pH, which explains the different results. Crude oil contains sodium, potassium chloride and sulfate, which increase electrical conductivity (EC) (Stahl and Williams, 1986). Hydrocarbon addition reduces the redox potential of soil from +833 mV to -982 mV (Ellis and Adams, 1961). A reduction in redox potential alters the solubility of metals; exchangeable iron and manganese, in particular, are higher in contaminated soil (Schollenberger, 1930; Udo and Fayemi, 1975). Adding hydrocarbons to soil increases the amount of both organic carbon (Adams and Ellis, 1960) and nitrogen (Stahl and Williams, 1986). However, as hydrocarbons have more carbon than nitrogen, the C:N ratio increases (Udo and Fayemi, 1975). Udo and Fayemi (1975) note that extractable phosphorus decreases with hydrocarbon addition. Micronutrients like magnesium and calcium increase with hydrocarbon addition (Stahl and Williams, 1986).

Industrial disturbance affects pH, electrical conductivity and fertility. Soil disturbance associated with pipeline (Naeth et al., 1987), and wellsite (Hammermeister, 2001) construction raised soil pH. Electrical conductivity increases in disturbed Solonetzic soils (Naeth et al., 1987; Rowell and Florence, 1993) due to an increase in salt sulphates that result from horizon mixing. However, Hammermeister (2001) found that EC on Chernozemic soils were not altered by well site disturbance. Both percentage organic carbon and total nitrogen are lower in disturbed compared to

undisturbed soils (Naeth et al., 1987; Hammermeister, 2001). The low fertility of disturbed soil is due to soil horizon mixing, which dilutes the quantity of soil nutrients (Hammermeister, 2001).

2.4 Effects of Hydrocarbons and Industrial Disturbance on Plants

2.4.1 Direct effects

Hydrocarbons act as herbicides, killing plants outright or causing extensive tissue damage. The effect on a plant varies with the properties of the hydrocarbon. The three main hydrocarbon classes have different toxicities, with alkanes being the least toxic, aromatics the most, and cycloalkanes in between (Baker, 1970). Within each class, smaller molecules are generally more toxic than larger ones (Baker, 1970). Furthermore, hydrocarbon phytotoxicity increases with higher temperatures and humidity, and drought conditions (Baker, 1970). Foliar application of crude oil results in the death of black spruce (*Picea mariana* (Mill.) BSP.), moss and lichens (Racine, 1994). Once inside plant tissue, hydrocarbons reduce transpiration, decrease photosynthesis and inhibit translocation (Baker, 1970).

Application of hydrocarbons to soil is not as damaging to plants as foliar exposure (Racine, 1994). This is because roots are more resistant than leaves (Currier and Peoples, 1954). In fact, some plants tolerate soil with up to 10% (wt/wt) crude oil (Radwan et al., 1995). However, beyond 3% (wt/wt) hydrocarbons in soil, most plants exhibit leaf chlorosis and necrosis, dehydration, stunted growth and shoot apex death (Baker, 1970; Udo and Fayemi, 1975; Xu and Johnson, 1995). Hydrocarbons in soil coat plant roots, which prevents water (Xu and Johnson, 1997) and oxygen (Udo and

Fayemi, 1975) uptake. Hydrocarbons also disrupt the geotropic orientation of plants possibly due to the resemblance of some crude oil components to plant growth hormones (Bossert and Bartha, 1985). Seeds planted in hydrocarbon-contaminated soil have reduced and delayed germination (Murphy, 1928; Baker, 1970; Amakiri and Onofeghara, 1984). This is because either oil penetrates the seed coat and kills the embryo, or coats the seed, preventing gas and water uptake (Baker, 1970).

2.4.2 Indirect effects

Both hydrocarbon addition and industrial disturbance affect soil properties, and the health and abundance of symbiotic microorganisms. Plant germination and growth is negatively affected by adverse soil conditions. The absence of symbiotic organisms may prevent germination and inhibit plant growth, especially among plants that are highly dependent on symbiotic microorganisms.

Reduced yield is observed when plants are grown on hydrocarbon-contaminated soil (Amadi et al., 1993; Chaîneau et al., 1996; Reilley et al., 1996). Rates as low as 0.5 % (wt/wt) crude oil reduce yields of field pea and barley (Xu and Johnson, 1995). Low yield is mainly due to low nitrogen availability in hydrocarbon-contaminated soil (Giddens, 1976). Shoot biomass decreases more than root biomass in hydrocarbon-contaminated soil (Xu and Johnson, 1997; Walton et al., 1994), as the typical response of a plant to nutrient and water stress is to increase root biomass (Fitter and Hay, 1987). At low concentrations, the presence of hydrocarbons may improve plant growth. Soybean growth increases when 0.75% (wt/wt) crude oil is added to a sandy peat soil (Carr, 1919). The addition of a 0.1% concentration of a mixture of organic chemicals

improves the growth of white clover, tilesy sage (*Artemisia tilesii* Ledeb.), Bering hairgrass (*Deschampsia beringensis* Hulten) and alpine bluegrass (*Poa alpina* L.) (Rogers et al., 1996). Improved plant growth occurs when hydrocarbons are converted into stable humus, which increases the organic carbon content of the soil (Toogood, 1977; Amadi et al., 1994). However, Abujnah (1999) cautions that fertilizer application may be required to facilitate the conversion of oily waste carbon to humus. Arid and saline conditions, which sometimes occur at hydrocarbon-contaminated sites, inhibit plant root growth (Gregory, 1987).

On disturbed soil, like mine tailings (Redente et al., 1982; Skousen et al., 1994) or sites with topsoil removed (Campbell and Souster, 1982; Verhagen et al., 2001), plant productivity is low compared to undisturbed soil. However, productivity may remain low on disturbed soil even when fertilizer and water are added (Ross et al., 1982). This implies that a change in soil structure has a negative effect on plant growth. The higher bulk density typically observed in disturbed soils restricts root penetration, impeding plant growth (Naeth et al., 1987).

Soil disturbance and the presence of hydrocarbons may negatively affect plant growth by killing symbiotic microorganisms or decreasing colonization. Vesicular-arbuscular (VA) mycorrhizal colonization decreases in field soils polluted with hydrocarbons (Stahl and Williams, 1986; Cabello, 1997; Leyval and Binet, 1998). However, spore numbers are lower in hydrocarbon-contaminated soil for some VAM species but not others, suggesting that hydrocarbon tolerance varies among species of fungi (Stahl and Williams, 1986). Leyval and Binet (1998) note that contaminating soil with 10 g/kg anthracene does not affect mycorrhizal colonization of leek (*Allium*

porrum L.), corn, ryegrass, and clover (*Trifolium subterraneum* L.). Soil disturbance reduces spore numbers and damages the soil hyphal network, which results in a decrease in mycorrhizal infectivity (Jasper et al., 1989; Moorman and Reeves, 1979). Miller (1978) notes that the majority of plants on old spoil piles in Wyoming are non-mycorrhizal, even though mycorrhizal spores are present in the soil. This is not surprising since the presence of spores is not correlated with mycorrhizal formation; most colonization is due to the presence of pre-existing hyphae in the soil (Brundrett, 1991). Less than 1% of plant cover on an old roadbed in Colorado was from mycorrhizal plants compared to 99% on adjacent undisturbed land (Reeves et al., 1979). Non-mycorrhizal plants may be competitively superior to mycorrhizal plants on disturbed land (Reeves et al., 1979). Numbers of mycorrhizae decline at sites with high salinity (Kim and Webber, 1985). Mycorrhizae are also less common on arid sites (Brundrett, 1991). Since soil disturbance and hydrocarbon contamination may affect the EC and water-holding capacity of the soil, mycorrhizal formation may be inhibited.

While some *Rhizobium* spp. inhabit hydrocarbon-contaminated soil (Ahmad et al., 1997), their ability to form nodules is suppressed (Stahl and Williams, 1986; Suominen et al., 2000). The cause of nodulation reduction is thought to be massive anthocyanin production by plants in the presence of PAHs like fluoranthene, which reduces the synthesis of nod-gene inducers (Wetzel and Werner, 1995). Nodulation is also affected by soil factors that change with the addition of hydrocarbons, like pH (Cheng et al., 2002). Even when nodules do form, nitrogenase activity is reduced in hydrocarbon-contaminated soil (Stahl and Williams, 1986).

The presence or absence of microorganisms may affect plant community structure, not just growth. Adding symbiotic microorganisms to soil used in plant competition experiments prevents the dominance of some plants and excludes others (St. John and Coleman, 1982; Chanway et al., 1991). Newman (1978) notes four ways that microorganisms alter plant community composition: (i) a microorganism may favour one plant species over another; (ii) a plant species may affect the microorganisms in the rhizosphere of another plant species, altering the plant's performance; (iii) rhizosphere microorganisms may detoxify allelochemicals produced by plants; and (iv) mycorrhizae may transfer nutrients between competing plant species. The result of competition between species with different mycorrhizal strategies is affected by soil properties (Brundrett, 1991). If phosphate is limiting, mycorrhizal plant species are favoured, but if water is limiting, non-mycorrhizal or facultatively mycorrhizal plant species prevail (Brundrett, 1991). Microbial communities may be driving succession (Francis and Read, 1994; Hart et al., 2001) favouring certain plant species over others.

2.5 Factors Affecting Plant Tolerance of Hydrocarbons

Plants in certain families may possess characteristics that make them more tolerant of hydrocarbons. Baker (1970) notes that succulent plants are resistant to hydrocarbons sprayed on their foliage, due to a thick cuticle and few stomata through which hydrocarbons can enter. Plants in the Apiaceae family, like carrot, are more tolerant of hydrocarbons sprayed on foliage due to the unique structure of the plasma membrane (Baker, 1970). Whether plants in these families are more resistant to

hydrocarbons in soil has not been explored. Gudin and Syratt (1975) suggest that legumes are more tolerant of hydrocarbons as they can fix nitrogen. However, legumes tested for tolerance and hydrocarbon degradation do not survive in contaminated soil as long as grasses (Kulakow et al., 2000); this may be due to a lack of active nodules.

One factor that may affect hydrocarbon tolerance in plants is seed size. As contaminated soils initially have high C:N ratios (Chaîneau et al., 1996), nitrogen may be unavailable to plants for some time. Several studies note that larger seeded species are more tolerant of soils low in nutrients than small seeded species (Jurado and Westoby, 1992; Allsopp and Stock, 1995; Kolawole and Kang, 1997), likely because their seed reserves can support growth for many weeks in the absence of external nutrients. However, this pattern is not always observed; Maranon and Grubb (1993) note that among Mediterranean annuals those with the largest seeds grow in habitats with a rich nutrient supply and those with smaller seeds in nutrient poor habitats.

Another factor that may affect tolerance is relative growth rate (RGR). Many studies note that plants with low RGRs are more tolerant of infertile soil than plants with high RGR (Rorison, 1968; Grime and Hunt, 1975; Chapin, 1980). The exact reason for this has not been determined. Grime and Hunt (1975) suggest it may be due to lower nutrient absorption by plants with low RGR. However, this hypothesis does not seem likely since a neighboring plant with higher nutrient absorption would capture the nutrients before the plant with lower absorption (Lambers and Poorter, 1992). There is some evidence that slow growing species store nutrients in vacuoles (Specht and Groves, 1966). The stored nutrients can be used when soil nutrients are exhausted, increasing the probability the plant will survive to the next nutrient flush (Chapin,

1980). However, this appears to be true only for phosphate, not other nutrients (Lambers and Poorter, 1992). Lambers and Poorter (1992) suggest that it is not a low RGR *per se* that gives a species an ecological advantage in infertile soils, but that it is one of the components correlated with RGR that is the target of selection. They hypothesize that plants with low specific leaf area (SLA) (i.e. amount of leaf area per unit leaf weight), a trait highly correlated with RGR, are better adapted to infertile soil. Plants with low SLA have low leaf turnover, which improves nutrient conservation (Aerts and Berendse, 1989; Berendse and Elberse, 1989). Regardless of the exact cause, the correlation between performance in infertile soils and RGR is well documented (Grime, 1979).

Grime (1979) defined stress as "the external constraints which limit the rate of dry matter production of all or parts of the vegetation." Chapin (1980) suggests that those plants facing any kind of stress (i.e. drought, salinity) will grow slowly and share many characteristics of plants adapted to infertile soil. Grime (1979) describes these plants as having a stress-tolerant strategy. Field experiments indicate that two or more resources may simultaneously limit plant growth, as plants possess homeostatic capabilities that reduce imbalances in resource requirements (Chapin et al., 1987). Grassland plants respond to either nitrogen or water addition (Lauenroth et al., 1978) and tundra plants to changes in temperature, light or nutrients (Chapin and Shaver, 1985). Since hydrocarbons are a cause of stress that affects the availability of nutrients and water, it is possible that plants adapted to infertile or arid habitats will also be able to tolerate hydrocarbon-contamination in soil. Some evidence already exists that this is indeed true. Weinstein and Yanai (1994) note that tolerance to ozone is higher in plants

with low RGRs. Plants growing on sites rich in heavy metals also have low RGRs (Wilson, 1988; Verkleij and Prast, 1989). In fact, Lasat (2002) states that slow growth of metal-tolerant plants hinders phytoremediation of metals. To date, the effect that seed size, RGR and SLA have on plant growth in hydrocarbon-contaminated soils has not been examined.

2.6 Methods of Selecting Plants for Phytoremediation

There are three methods used to screen plants for phytoremediation potential: (1) document plants that naturally grow on contaminated sites, (2) grow plants in contaminated soil under greenhouse/growth chamber conditions, and (3) grow plants in contaminated soil under field conditions. No one method has been accepted as a standard. Some researchers suggest that the plant screening process should involve several stages (Liste and Alexander, 1999; Olson and Fletcher, 2000). For example, Olson and Fletcher (2000) suggest that the examination of vegetation at field sites should be the first level of screening, with controlled experiments conducted using promising species to evaluate degradation abilities.

There are several studies in which plant abundance and composition at hydrocarbon-contaminated sites in the field are documented. One study involves the examination of a former industrial sludge basin where water was drained (Olson and Fletcher, 1999). Thirteen years after drainage plant cover on the former sludge basin is the same as that on nearby uncontaminated sites. However, mulberry (*Morus* spp. L.), Bermuda grass (*Cynodon dactylon* L. Pers.) and annual sunflower (*Helianthus annuus* L.) are much more common on the hydrocarbon-contaminated site than on the

uncontaminated sites (Olson and Fletcher, 1999). Legumes, particularly black medick (*Medicago lupulina* L.) and bird's-foot trefoil (*Lotus corniculatus* L.) are the dominant plants on 15 oil-contaminated sites in Europe (Gudin and Syratt, 1975). In Colorado, umbrellaplant (*Eriogonum corymbosum* Benth.), a half shrub, is more common on spent oil shale than on control sites (Mackey and Depuis, 1985). Vegetation recovery studies after experimental crude oil spills in the arctic note that green alder (*Alnus crispa* (Ait.) Pursh), bog birch (*Betula glandulosa* Michx.), willow (*Salix* spp. L.), Labrador tea (*Ledum groenlandicum* Oeder), bog cranberry (*Vaccinium vitis-idaea* L.), bog bilberry (*V. uliginosum* L.), marsh horsetail (*Equisetum palustre* L.), dwarf scouring rush (*E. scirpoides* Michx.) (Hutchinson and Freedman, 1978), woodland horsetail (*E. sylvaticum* L.) and sheathed cotton-grass (*Eriophorum vaginatum* L.) (Racine, 1993) are particularly tolerant. Although documenting natural revegetation helps identify tolerant species, individual plant performance with regards to degradation ability is not assessed.

For greenhouse/growth chamber studies two types of soils are used: (1) soil contaminated in the field (Rogers et al., 1996; Wiltse et al., 1998) and (2) soil to which contaminants are added just prior to experimentation (Reilley et al., 1996; Kulakow et al., 2000). The type of soil used may affect the outcome of the experiment, as field-contaminated soils often have altered physical and chemical properties due to industrial disturbance (section 2.3).

Unfortunately, no one has compared plant germination and growth in field contaminated soil with that attained when hydrocarbons are added to soil prior to greenhouse studies. Both plant growth parameters and the effect of plants on contaminant concentration are used to select plants for phytoremediation (Reilley et al.,

1996; Rogers et al., 1996; Wiltse et al., 1998; Kulakow et al., 2000). Germination, maturity, height, shoot and root dry weight, root to shoot ratio, root diameter and root length density are the plant growth parameters that have been used to identify cold-tolerant phytoremediators (Rogers et al., 1996; Wiltse et al., 1998; Kulakow et al., 2000). Sometimes seeds are planted in the contaminated soil (Rogers et al., 1996; Kulakow et al., 2000) and sometimes seedlings (Reilley et al., 1996). Liste and Alexander (1999) do not even use contaminated soil to screen plants; instead 14-day old seedlings are placed in tubes of deionized water and phenanthrene, and after one hour, phenanthrene remaining in solution is measured. Amakiri and Onofeghara (1984) simply test plants for their ability to germinate in Petri dishes after exposure to crude oil.

One problem with greenhouse experiments is that significant reduction of the contaminant may not have occurred during the time allotted for the study, especially if the soil contains recalcitrant chemicals (Kulakow et al., 2000). For example, differences in TPH concentrations among alfalfa varieties are detected after 12 months but not six (Wiltse et al., 1998). Another problem with greenhouse/growth chamber studies is that plants are rarely exposed to the types and variability of climatic conditions that are encountered in the field. The freshly contaminated soil often used in these experiments is more toxic than weathered field soils where many of the hydrocarbons have volatilized or adsorbed to organic matter and clay. Collecting contaminated field soil and using it for greenhouse experiments may be more realistic than using freshly contaminated soil. However, once contaminated soil is removed

from the field site, hydrocarbon volatilization and soil oxygen would likely increase, resulting in different degradation rates than under field conditions.

Field studies involve planting seeds or adult plants in contaminated soil. These types of studies are more realistic than greenhouse/growth chamber studies as the plants are exposed to normal environmental conditions. However, very few phytoremediation field studies have been conducted to date. Verde kleingrass (*Panicum coloratum* L. var. 'Verde') is considered a promising phytoremediator based on its visual appearance, root length and PAH concentration reduction when grown in clay soil (Qui et al., 1997). Common buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) significantly reduces naphthalene concentrations in the field (Qui et al., 1997). Transplanting sod of cordgrass (*Spartina alterniflora* Loisel) and salt meadow grass (*S. patens* (Ait.) Muhl.) into a contaminated wetland enhanced the oil degradation rate, more so when fertilizer was added as well (Lin and Mendelssohn, 1998). One of the problems with assessing phytoremediation success in the field is that contaminants are unevenly distributed throughout the soil (Qui et al., 1987). Many replicates have to be sampled to accurately assess degradation.

3.0 IMPACT OF PETROLEUM HYDROCARBONS ON THE SOIL AND PLANT COMMUNITIES OF SOUTHERN SASKATCHEWAN

3.1 Abstract

Phytoremediation as a technique to remediate contaminated soil is gaining popularity due to low cost and minimal soil disturbance. A suggested way to identify hydrocarbon-tolerant species for phytoremediation is to sample the vegetation at contaminated sites allowed to recover naturally. We documented the plant species that naturally colonized 14 hydrocarbon-contaminated plots in southern Saskatchewan, and compared it to those on nearby uncontaminated plots. The most common species on contaminated soil, particularly on soils that were disturbed, were the annual forb kochia (*Kochia scoparia* (L.) Schrad.) and the perennial grass wild barley (*Hordeum jubatum* L.). On contaminated soils with high clay contents and electrical conductivities, the native grasses salt grass (*Distichlis stricta* (Torr.) Rydb.) and Canby bluegrass (*Poa canbyi* (Scribn.) Piper) were the most common species. The native grasses western wheatgrass (*Agropyron smithii* Rydb.) and slender wheatgrass (*A. trachycaulum* var. *trachycaulum* (Link) Malte), had similar cover and frequency on contaminated and uncontaminated soil.

The soils of contaminated plots had significantly higher pH and carbon to nitrogen ratios, and lower nitrogen and phosphorus concentrations. Contaminated plots had significantly less vegetation and litter cover than uncontaminated plots. Although

species richness was not significantly different between contaminated and uncontaminated plots, diversity was significantly lower on contaminated plots. All contaminated plots, except one, were less than 50% similar to the uncontaminated plots in species richness and all plots, except two, were less than 30% similar in species abundance. Species that were non-native, non-mycorrhizal, annual, biennial, large-seeded and that reproduced by seed were more common on contaminated plots. Furthermore, species that were non-mycorrhizal, self-pollinated, large-seeded and that reproduced by seed formed more plant cover on contaminated plots, while woody species and those with unassisted or bird-dispersed seeds formed less.

3.2 Introduction

As hydrocarbon-contaminated sites occur throughout Canada, and are threats to human and ecosystem health, remediation techniques are needed. Phytoremediation is the use of plants and their associated microorganisms to degrade, contain or sequester soil contaminants (Cunningham et al., 1996). Plants improve hydrocarbon degradation rates in soil by providing hydrocarbon-degrading microorganisms with compounds that improve growth or accelerate degradation (Cunningham et al., 1996). Phytoremediation is gaining in popularity due to low cost and minimal soil disturbance relative to engineering and bioremediation techniques (Germida et al., 2002). One of the barriers to implementation of phytoremediation biotechnology is that only a few species capable of growing in Canada have been identified as potential phytoremediators.

Oil exploration has a variety of negative effects on vegetation, both direct and indirect. Spilled oil kills plants, decreases and delays seed germination, and causes leaf

chlorosis (Baker, 1970; Udo and Fayemi, 1975; Chaîneau et al., 1996). Oil spills increase soil C:N ratio, immobilize nitrogen and reduce plant yield (Xu and Johnson, 1997). The hydrophobicity of contaminated soil causes plant dehydration (Udo and Fayemi, 1975). The ability of mycorrhizae and nitrogen-fixing bacteria to infect plants decreases in hydrocarbon-contaminated soil (Cabello, 1997; Leyval and Binet, 1998; Suominen et al., 2000). Soil disturbance at contaminated sites also decreases mycorrhizal activity (Miller 1978; Jasper et al., 1989), and results in exposure of the B horizon or horizon mixing (Rowell and Florence, 1993). Soil compaction occurs at many contaminated sites due to vehicular and human traffic (Rowell and Florence, 1993). In certain parts of western Canada, oil and gas deposits are sealed by salt caprock (Storer, 1989). During drilling, salt water is sometimes spilled along with oil, causing salinization. Therefore, plants to be used for phytoremediation must not only tolerate hydrocarbons but also other adverse conditions that occur at contaminated sites.

Species that naturally colonize hydrocarbon-contaminated sites can tolerate adverse field conditions, so conducting surveys of these sites can aid in the selection of appropriate species for phytoremediation. Surveys in the United States have identified a few species native to western Canada that are tolerant of hydrocarbons. Sunflower (*Helianthus annuus* L.) is particularly abundant on a former industrial sludge basin in Texas (Olson and Fletcher, 2000). Saskatoon berry (*Amelanchier alnifolia* Nutt.) is one of the dominant species on spent oil shale sites in Colorado (Mackey and DePuit, 1985). Vegetation colonizing disturbed but uncontaminated sites like abandoned oil wells and coal mine spoils, may also colonize hydrocarbon-contaminated sites, as the types of soil disturbances (i.e. horizon mixing and compaction) may be similar. Hammermeister

(2001) noted that northern wheatgrass (*Agropyron dasystachyum* (Hook.) Scribn.), western wheatgrass, blue grama (*Bouteloua gracilis* HBK. Lag.), sedge (*Carex* spp. L.), little club moss (*Selaginella densa* Rydb.) and speargrass (*Stipa comata* Trin. & Rupr.) are dominant on abandoned, uncontaminated well sites in Alberta. In southern Saskatchewan non-native annuals like kochia and Russian thistle (*Salsola kali* L.) are common on coal mine spoil less than five years old (Jonescu, 1979). Slender wheatgrass, pasture sage (*Artemisia frigida* Willd.), many-flowered aster (*Aster ericoides* L.), gumweed (*Grindelia squarrosa* (Pursh) Dunal), wild barley, yellow sweet-clover (*Melilotus officinalis* (L.) Lam.), perennial sow-thistle (*Sonchus arvensis* L.) and goat's-beard (*Tragopogon dubius* Scop.) are the most common perennial species colonizing older coal mine spoil (Jonescu, 1979). Surveys of abandoned coal mines in North Dakota also found that annuals like kochia are common for the first few years but that longer lived perennial species like western wheatgrass, prairie sage (*Artemisia ludoviciana* Nutt.), many-flowered aster and wild barley eventually dominate (Iverson and Wali, 1982; Wali, 1999).

Attempts have been made to determine if plants possessing certain functional attributes are more common on hydrocarbon-contaminated than on uncontaminated soil. Legumes are abundant on hydrocarbon-contaminated sites in Europe likely due to their nitrogen-fixing ability (Gudin and Syratt, 1975). The ability of mycorrhizae to infect plants decreases in hydrocarbon-contaminated soil (Cabello, 1997; Leyval and Binet, 1998; Suominen et al., 2000). On disturbed sites of low fertility, herbaceous species are more common than woody species (Tilman, 1987; Skousen et al., 1994). Soils with low fertility are purported to have a greater proportion of forbs than grasses (Hobbs et al.,

1988). Wind-pollinated and -dispersed plants are thought to be more common on disturbed areas than animal-pollinated and -dispersed ones (Johnson, 1981). Early successional species often reproduce solely by seed (Wali, 1999; Karel et al., 2001). Several studies have found that plants with large seeds are more common on infertile soil (Kolawole and Kang, 1997; Milberg et al., 1998). If species with particular functional or structural attributes are more common on contaminated than surrounding uncontaminated land, other species with the same attributes may also be successful in colonizing contaminated land.

The objective of this study was to identify which plant species in the Aspen Parkland and Mixed Grassland Ecoregions of Saskatchewan grow on unrefined hydrocarbon-contaminated soils. Vegetation on contaminated soil was compared to that on adjacent uncontaminated soil. The effect contamination had on percentage cover, litter and bare ground was also examined. Differences in species composition between contaminated and uncontaminated plots were assessed using similarity indices. The plant family, origin, ability to fix nitrogen and form mycorrhiza, life form, pollination mode, seed dispersal mechanism, reproduction mode and seed size was determined to see if species with certain attributes were more common on contaminated than uncontaminated soil. Contaminated plots without adjacent native prairie were excluded from the statistical analyses to determine if this influenced species and functional group composition and abundance.

3.3 Materials and Methods

3.3.1 Study sites

In August, 2001 vegetation and soils were sampled at 14 sites in the Aspen Parkland and Mixed Grassland Ecoregions in Saskatchewan, Canada (Table 3.1). The climate is arid with a cold season; mean annual precipitation is 380 mm and mean annual temperature 3.2 °C (Coupland, 1950). Parent material is glacial in origin, mainly till or lacustrine deposits (Acton et al., 1998). All soils were in the Chernozemic order but they were in five different subgroups and had widely differing textures (Table 3.2). The study sites occurred over a range of habitats: hills, flats, saline wetlands and stabilized sand dunes.

All contaminated plots except Cantuar, Fosterton and Success 3 had at least several hectares of native grassland or woodland either completely surrounding it or adjacent to it from which propagules of native species could have arrived. All surface spills surrounded decommissioned oil wells but several buried flare pits (i.e. excavated soil pits where petroleum waste was deposited and natural gas burned off) were also examined (Table 3.1). Because contamination with unrefined petroleum hydrocarbons occurred over a period of several years at some sites and the exact age of the spill(s) was not always known, contamination age was classified into categories (i.e. 1-5, 5-10 or 30-40 years). The level of human disturbance varied from site to site. The Cantuar site had been cultivated the year before. At Fosterton, Forget and Winter 1 and 3 some soil had been excavated and mechanically removed from the site with a bobcat in 2000 as part of ongoing site reclamation. Soils of the contaminated plots at Arcola 1 and 2 and Hassard were compacted by pickup trucks that traveled to the sites for well

Table 3.1. Location, ecological and soil classification, habitat and contamination information of the 14 sites sampled in southern Saskatchewan.

Site	Legal Land Description	Ecoregion [a]	Ecodistrict [b]	Chernozemic Soil Subgroup	Habitat	Location of Native Prairie [c]	Type of Contamination	Time [d] (yrs)
Forget	21-10-7-W2	AP [e]	Gainsborough Plain	Orthic Black	Hilly native prairie	Adjacent	Surface spill	1-5
Manor 1	12-6-1-W2	AP	Gainsborough Plain	Gleyed Black	Saline wetland	Adjacent	Surface spill	1-5
Manor 2	14-6-1-W2	AP	Gainsborough Plain	Gleyed Black	Saline wetland	Adjacent	Surface spill	1-5
Arcola 1	26-7-4-W2	AP	Gainsborough Plain	Orthic Black	Native prairie flat	Adjacent	Surface spill	5-10
Arcola 2	16-9-4-W2	AP	Moose Mountain	Orthic Black	Hilly native prairie	Surrounding	Surface spill	5-10
Hassard	24-9-5-W2	AP	Moose Mountain	Orthic Black	Hilly native prairie	Surrounding	Surface spill	1-5
Winter 1	31-42-25-W3	AP	Ribstone Plain	Rego Black	Stabilized sand dune	Surrounding	Surface spill	5-10
Winter 2	31-42-25-W3	AP	Ribstone Plain	Rego Black	Stabilized sand dune	Surrounding	Surface spill	5-10
Winter 3	31-42-25-W3	AP	Ribstone Plain	Rego Black	Stabilized sand dune	Surrounding	Surface spill	5-10
Fosterton	10-17-18-W3	MG [f]	Antelope Creek Plain	Orthic Brown	Native prairie flat	Within 1.6 km	Buried flare pit [g]	30-40
Cantuar	26-16-16-W3	MG	Gull Lake Plain	Orthic Brown	Cultivated flat	Within 1.6 km	Surface spill	1-5
Success 1	9-17-16-W3	MG	Gull Lake Plain	Gleyed Brown	Saline wetland	Adjacent	Buried flare pit	30-40
Success 2	9-17-16-W3	MG	Gull Lake Plain	Gleyed Brown	Saline wetland	Adjacent	Buried flare pit	30-40

Table 3.1, continued

Site	Legal Land Description	Ecoregion [a]	Ecodistrict [b]	Chernozemic Soil Subgroup	Habitat	Location of Native Prairie [c]	Type of Contamination	Time [d] (yrs)
Success 3	10-17-16-W3	MG	Gull Lake Plain	Orthic Brown	Native prairie flat	Within 1.6 km	Buried flare pit	30-40

[ab] Based on Acton et al., 1998

[c] Indicates if native prairie surrounded, was adjacent to, or within 1.6 km of the contaminated plot

[d] Elapsed time since initial contamination

[e] AP = Aspen Parkland

[f] MG = Mixed Grassland

[g] Unlined soil pits used to store and/or burn produced fluids such as liquid hydrocarbons, process chemicals, crude bitumen or salt water, and filled with soil

Table 3.2. Soil characteristics for 0-15 cm depth at the 28 uncontaminated and contaminated plots sampled in southern Saskatchewan.

	Texture Class [a]		pH		Electrical Conductivity (mS/cm)		Total Carbon (%)		Total Nitrogen (%)		C:N Ratio		Available PO_4^{3-} (µg/g)		Available K (µg/g)		TPH [b] (µg/g)
Site	U [c]	C [d]	U	C	U	C	U	C	U	C	U	C	U	C	U	C	C
Forget	L	SCL	7.2*	8.3	0.17*	0.74	4.52	3.06	0.41	0.12*	11:1	26:1	1.0	1.0	316	190*	232
Manor 1	SL	SL	7.6	7.9	13.70	6.44**	9.40	2.65***	0.82	0.13**	11:1**	21:1	0.2*	1.4	219	321	379
Manor 2	L	SCL	7.8	7.6	6.21***	17.20	5.75*	8.71	0.43	0.69	13:1	13:1	0.6	1.0	832	346*	142
Arcola 1	LS	LS	8.3	8.4	0.29***	0.51	8.43*	3.01	0.54	0.08	16:1	38:1	1.2	0.7	261	194*	112
Arcola 2	SL	SL	7.4*	8.2	0.30	0.25	4.02	3.38	0.24	0.17*	17:1*	20:1	0.1*	1.2	278	364	64
Hassard	L	CL	7.8	8.0	0.23	0.21	3.29	2.83	0.17	0.06	19:1*	51:1	2.6	1.6	237	304	140
Winter 1	CL	SCL	8.1	7.9	0.61*	1.47	7.53	6.98	0.06	0.30	17:1	23:1	2.2	1.9	476**	943	237
Winter 2	SL	LS	7.4*	8.1	0.25	0.24	8.33	1.16*	0.43	0.06	19:1	19:1	6.8	1.8*	296	294	43
Winter 3	SL	SL	8.1	8.0	0.78	0.97	3.71*	7.72	0.25*	0.48	15:1	16:1	5.4	3.6*	367*	560	353
Fosterton	SiL	L	7.4*	7.9	0.88	0.25*	3.52	2.60*	0.27	0.11*	13:1**	24:1	13.7	1.0*	418	338*	148
Cantuar	SiL	L	7.3	7.6	0.60***	5.07	2.88**	5.34	0.15	0.14	20:1*	39:1	10.5	1.8*	448	186*	316
Success 1	SiC	CL	7.6	7.7	11.29	4.91**	4.28	4.06	0.22	0.11	19:1	36:1	1.2*	2.3	369	323*	474

Table 3.2, continued

	Texture Class [a]		pH		Electrical Conductivity (mS/cm)		Total Carbon (%)		Total Nitrogen (%)		C:N Ratio		Available PO_4^{3-} (µg/g)		Available K (µg/g)		TPH [b] (µg/g)
Site	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	C
Success 2	SiCL	CL	7.6*	8.1	2.01	18.50	4.09	3.82	0.25	0.08**	16:1***	47:1	0.0*	0.8	446	280*	383
Success 3	SiCL	SiCL	7.3	7.7	0.76**	2.94	9.28	7.72	0.65	0.16**	14:1*	33:1	12.6	2.0**	214*	348	496
Mean	N/A	N/A	7.6**	8.0	2.72	4.26	5.65	4.50	0.35	0.19**	16:1***	29:1	4.2	1.6*	370	357	251

[a] C = Clay; L= Loam; S = sand; Si = Silt

[b] TPH = Total Petroleum Hydrocarbons

[c] U = Uncontaminated plot

[d] C = Contaminated plot

*, **, *** Significantly higher than adjacent plot at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively using Student's t-test

maintenance. The size of the spills was less than 1 ha, the largest being the buried flare pits. None of the contaminated plots had been grazed by cattle but the uncontaminated plots at Forget, Manor 1, Hassard, Winter 3, Success 2 were. The intensity and duration of the grazing at these sites was not known.

3.3.2 Soil and vegetation sampling

Two 400 m^2 plots were established at each site, one on contaminated and one on uncontaminated soil. The minimum distance between uncontaminated and contaminated plots was 20 m. The extent of the contamination was delineated by visually determining the edge of the associated soil and vegetation disturbance and situating the uncontaminated plots at least 10 m from it. Four randomly located 5 x 5 m subplots, at least 5 m apart, were established in each contaminated and uncontaminated plot (Figure 3.1). Soil from the 0 to 15 cm depth was collected with a hand shovel from the center of each subplot, mixed in a plastic bucket and 125 mL samples placed in sealed glass containers (EPA, Edmonton, AB #JC0125-24NC, CA63450-006). The soil samples remained at ambient (about 22 °C) temperature until they were stored within two days of collection in a refrigerator at 5 °C. The soils were passed through a 5 mm sieve to exclude rocks and clumps of tar prior to analysis, which occurred within three months of collection.

The four soil samples from each plot were analyzed for %C and %N using a LECO CNS-2000 Analyzer (LECO Corporation, Mississauga, ON). The petroleum hydrocarbons in a 1 g soil sample from each of the four subplots were extracted three

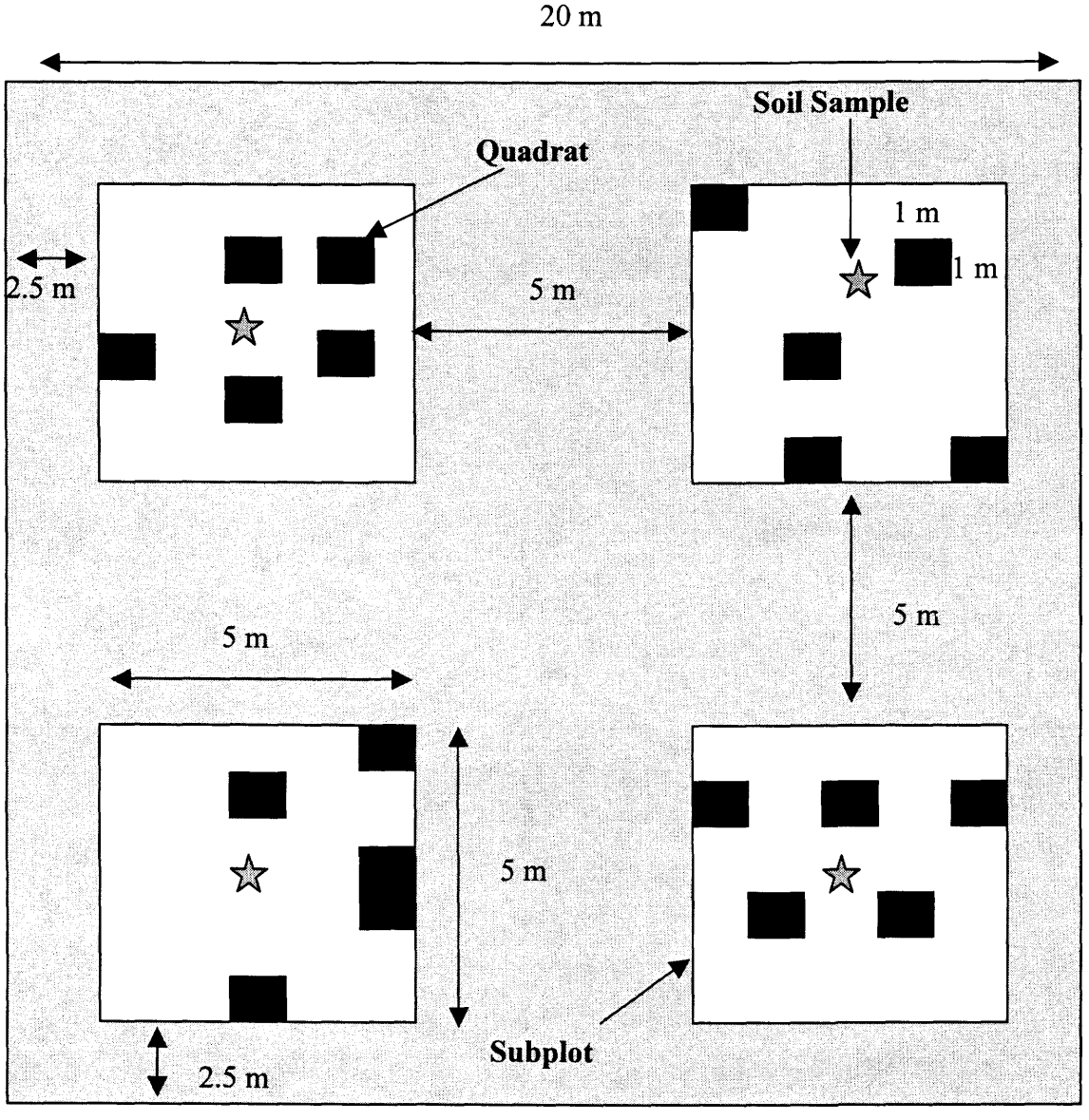

Figure 3.1. Size and shape of the four subplots and 20 randomly located quadrats, and location of soil samples at each plot.

times sequentially using a mechanical shaking extraction method with acetone as the solvent (Schwab et al., 1999). A 1 μL soil extract sample from each subplot was analyzed using a Hewlett-Packard 5890 gas chromatograph (GC) equipped with a flame-ionization detector and an HP1 capillary GC column. The helium carrier gas flow rate was 40 cc/minute. The column temperature was set at 40 °C for two minutes then ramped at 17 °C/minute to a final temperature of 275 °C. Five crude oil standards (i.e. 250, 500, 1,000, 1,250 and 2,500 ppm) were prepared by adding the appropriate quantity of crude oil to 20 mL acetone. By constructing a calibration curve from these standards, the GC areas were converted to concentrations (Figure 3.2). Soil from one subplot thought to be uncontaminated was in fact contaminated with hydrocarbons and was disregarded.

The remaining soil from the four subplots was mixed to form composite samples. Two replicates of this composite sample were analyzed for texture using the pipette method (Gee and Bauder, 1986), pH (McLean, 1982) and electrical conductivity (EC) using a 1:1 soil solution ratio (Rhoades, 1982), and available phosphorus (P) (Qian and Schoenau, 1994-1995) and available potassium (K) using anion and cation exchange membranes (Qian et al., 1996).

In August of 2001, absolute ground cover of plant species less than 1 m in height, canopy cover of plant species taller than 1 m, and ground cover of litter and bare soil were visually estimated in five randomly located 1 x 1 m quadrats per subplot (20 quadrats/plot) using Braun-Blanquet cover classes (Mueller-Dombois and Ellenberg, 1974). A 1 x 1 m quadrat size was chosen because it minimizes the perimeter to area ratio while still allowing easy observation of the entire quadrat (Barbour et al., 1987).

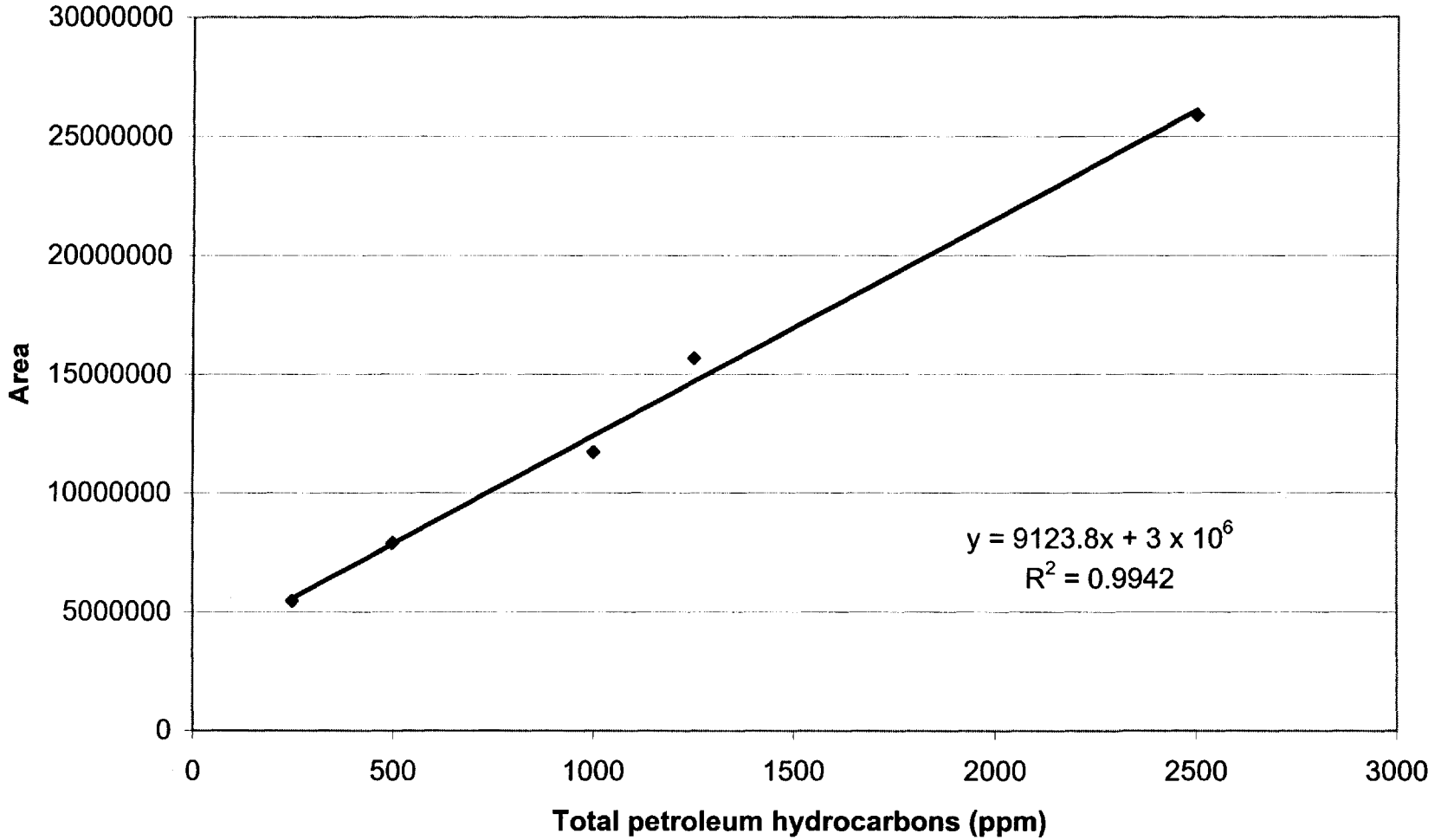

Figure 3.2. Total petroleum hydrocarbon calibration curve for contaminated field soils analyzed using a mechanical shaking extraction method and gas chromatograph.

Visual estimation of canopy cover was improved by using 10 cm^2 subquadrats when the cover class of a species was difficult to ascertain. Relative canopy cover data were used to calculate species richness (i.e. number of species per plot) and Shannon-Weiner diversity:

$$\text{Shannon-Weiner Diversity} = -\Sigma\, p_j \ln p_j \qquad [3.1]$$

where

$$p = \text{abundance of species j} / \Sigma \text{ abundance of all species} \qquad [3.2]$$

Frequency was determined by recording the number of quadrats each species was rooted in. Canopy cover of plants not rooted in the quadrat was included in the cover estimate but not frequency. The cover and frequency data are presented in Appendices C, D, E and F. Voucher specimens of each species encountered were collected and deposited in the W.P. Fraser Herbarium at the University of Saskatchewan. The roots of the voucher specimens were examined for evidence of growth into hydrocarbon clods and the species in which this occurred recorded. The taxonomy followed that of Moss (1983).

Because richness and diversity do not indicate which species two plots have in common, the similarity of the contaminated and uncontaminated plots was determined. Similarity indices provide mathematical expressions for the similarity of two plant communities (Mueller-Dombois and Ellenberg, 1974), and have been suggested as a way of evaluating recovery of revegetated mined land (Chambers, 1983). The species composition similarity of contaminated and uncontaminated plots were calculated:

$$\text{Jaccard's \% similarity index} = \frac{T}{C+U+T} x100 \qquad [3.3]$$

where C= the number of species found only in the contaminated plot, U = the number of species found in only in the uncontaminated plot, and T = the number of species found in both contaminated and uncontaminated plots. The similarity of species cover was examined:

$$\text{Spatz's \% similarity index} = Rx\frac{M_T}{M_C + M_U + M_T}x100 \qquad [3.4]$$

where M_C = sum of species cover values restricted to the contaminated plot, M_U = sum of species cover values restricted to the uncontaminated plot, and M_T = sum of species cover values found in both contaminated and uncontaminated plots and

$$R = \sum \frac{Sj^{c,u} / Sji^{c,u}}{S_T{}^{c,u}} \qquad [3.5]$$

where S_j = cover value of species j common to 'c' and 'u' that is smaller, and S_{ji}= cover value of species j common to 'c' and 'u' that is larger, S_T = total number of species, c = contaminated plot, and u = uncontaminated plot.

The ability of species found in the study plots to fix nitrogen and form mycorrhiza was determined from the literature (Currah and Van Dyk, 1986; Harley and Harley, 1987; Pahl and Smreciu, 1999). Origin (i.e. non-native or native), plant family, life form (i.e. woody or herbaceous perennial, biennial or annual), pollination mode (i.e. self, wind or insect), seed dispersal mechanism (i.e. wind, bird, mammal or unassisted) and reproduction mode (i.e. vegetative or by seed only) of each species was also determined from the literature (van der Pijl, 1972; Johnson, 1981; Moss, 1983; Fenner, 1985; Grime et al., 1990; Smith et al., 1997; Pahl and Smreciu, 1999). The seed size class each species belongs to was determined by weighing at least ten seeds randomly selected from seed lots collected in the field or obtained from the W.P. Fraser

Herbarium. The plant family and functional characteristics of each species are listed in Appendices A and B. The percentage of species and canopy cover attributable to species with these different functional attributes was determined.

3.3.3 Data analyses

Student's t-tests for soil characteristics and percentage cover between uncontaminated and contaminated subplots were performed. Student's t-tests of the soil characteristics, percentage cover, richness, diversity, and cover and frequency of principal species and functional groups between all uncontaminated and contaminated plots were performed. Three sites not surrounded or bordered by native prairie (e.g. Fosteron, Cantuar and Success 3) were excluded from the statistical analyses to determine if the presence of nearby native prairie affected species and functional group abundance. These statistical tests were done using MINITAB software (MINITAB Inc., State College, PA) with $P \leq 0.05$.

Ordination, a multivariate statistical technique that attempts to find a natural order among random quadrats, was used to further analyze the data (Pielou, 1984). The end result of an ordination is a multidimensional scatter diagram, which is then projected in two dimensions (Pielou, 1984). Any intrinsic pattern in the scatter diagram becomes apparent. The ordination axes represent environmental variables that affect the pattern. In general, the first three axes explain the greatest amount of the variation observed (Pielou, 1984).

There are two types of ordinations that differ in how axes are interpreted: indirect and direct gradient analyses. With indirect gradient analyses, like detrended

correspondence analysis (DCA), the interpretation of which environmental variables the axes represent is done by the analyst (Pielou, 1984). If the environmental variables relate strongly to the first few axes, indirect gradient analysis is easy and useful.

If environmental variables do not relate strongly to the first few axes, interpretation is more difficult. Direct gradient analyses, like Canonical Correspondence Analysis (CCA), directly relates plant community variation to environmental variation (Ter Braak, 1986). Due to the weak correlation of the environmental variables in this study as determined by conducting a DCA (data not presented), CCA was used to determine which variables most influenced the vegetation. This was done by imposing the extra restriction that the axes be linear combinations of environmental variables (Ter Braak, 1986). In the resulting ordination diagram, sites are represented by triangles and lines represent environmental variables. The length of the line indicates the strength of the correlation. By examining the signs and relative magnitude of the intraset correlations in the associated ordination table, the relative importance of each environmental variable can be determined. Percent soil carbon, nitrogen, silt, sand, clay, litter cover and bare ground, and electrical conductivity, pH, C:N ratio, total petroleum hydrocarbons, available phosphorus and potassium, soil zone and time since initial contamination were treated as environmental variables.

In some instances data must be transformed to prevent certain values from unduly influencing the analysis (Pielou, 1984). If data is highly skewed, it is recommended that it be transformed by relativization (Ter Braak, 1986). As the data for several variables particularly EC, available potassium and C:N ratio were highly skewed, both species percent cover and environmental variables were relativized by

species or environmental variable maximum. Axis scores were centered and standardized to unit variance and scaled to optimize the representation of plots (weighted mean scores for species cover). PCORD (MjM Software Design, Gleneden Beach, OR) was used to conduct the multivariate analyses.

3.4 Results

3.4.1 Soil properties

Soil characteristics at matched contaminated and uncontaminated plots were often different (Table 3.2). At nine sites the texture class was different between the matched plots. When comparing all the sites together, uncontaminated plots had significantly lower pH and C:N ratio, and higher total nitrogen and available phosphorus. The higher C:N ratio in contaminated soil was usually due to lower nitrogen rather than higher carbon. Electrical conductivity was significantly lower at three contaminated and six uncontaminated plots. Total carbon was significantly lower in four uncontaminated and three contaminated plots. Available potassium was significantly lower at seven contaminated and three uncontaminated plots. Total petroleum hydrocarbons were low, ranging from 64 to 496 ppm. The plots with the highest concentration of hydrocarbons also had among the highest C:N ratios (i.e. Success 1, 2 and 3). At Winter 1 and 3 fertilizer was added, resulting in a lower C:N ratio than would be expected given the hydrocarbon concentration. At the buried flare pit sites (i.e. Success 1, 2, 3 and Fosterton) patches of viscous hydrocarbons had reached the subsurface and surface. As these clods of hydrocarbons were not collected

as part of the soil sample, the actual hydrocarbon concentration at these plots is likely higher than reported here.

3.4.2 Plant communities

To determine which species were more common at contaminated plots, relative plant species cover and frequency at all 14 sites were determined (Table 3.3). Total graminoid cover was similar between the contaminated and uncontaminated plots. However, the graminoid with the greatest cover on contaminated plots was wild barley and on uncontaminated plots was Kentucky bluegrass (*Poa pratensis* L.). Wild barley and Kentucky bluegrass were also the most frequently encountered plants on contaminated and uncontaminated plots, respectively. However, only Kentucky bluegrass cover and frequency were significantly lower on contaminated compared to uncontaminated plots. Salt grass formed significantly more cover on contaminated than uncontaminated plots but was less frequent than some of the other graminoids as it was restricted to sites with high EC. Smooth brome (*Bromus inermis* Leyss.) had similar cover on contaminated and uncontaminated plots but a significantly lower frequency. The cover and frequency of the remaining grasses was not significantly different.

Forbs comprised significantly more cover on contaminated than uncontaminated plots with kochia making up 13% of the cover, slightly more than the amount contributed by all forbs on uncontaminated plots. Although cover of kochia was not significantly different between contaminated and uncontaminated plots, it was less frequent on uncontaminated plots. Only hairy golden aster (*Heterotheca villosa* (Pursh) Shinners) cover was significantly lower on contaminated plots. Although overall forb

Table 3.3. Percentage cover and frequency of the principal species and groups of species found on uncontaminated and contaminated plots based on 20 observations per plot at 14 field sites in southern Saskatchewan.

Species	Cover (%)		Frequency (%)	
	U [a]	C [b]	U	C
Agropyron pectiniforme [c]	2.3	6.6	5.7	12.1
Agropyron smithii	10.7	11.4	21.1	18.6
Agropyron trachycaulum	9.6	9.2	23.6	20.0
Bouteloua gracilis	3.0	0.0	5.0	0.4
Bromus inermis [c]	8.2	5.0	22.5	11.8*
Carex aquatilis	4.8	0.0	5.4	0.0
Distichlis stricta	4.6*	12.4	8.6	16.8
Hordeum jubatum	3.3	12.7	17.5	26.4
Koeleria macrantha	0.4	2.1	1.1	4.6
Poa canbyi	5.6	7.8	10.4	8.2
Poa pratensis [c]	19.9	7.5*	43.2	19.6*
Other graminoids	9.6	3.0	—	—
Total graminoids	***82.0***	***77.6***	—	—
Aster ericoides	0.8	0.5	19.6	15.7
Cirsium arvense [c]	0.9	0.4	13.2	10.4
Grindelia squarrosa	0.1	1.5	9.6	16.8
Heterotheca villosa	3.0	0.2*	8.6	7.1
Kochia scoparia [c]	0.4	13.0	3.2*	26.1
Other forbs	7.9	6.5	—	—
Total forbs	***12.5****	***22.3***	—	—
Rosa arkansana	0.3	<0.1	0.4*	10.7
Symphoricarpos occidentalis	4.1	<0.1*	1.1*	17.5
Other shrubs	1.1	0.2	—	—
Total shrubs	***5.5***	***0.2****	—	—

[a] U = Uncontaminated
[b] C = Contaminated
[c] Indicates non-native species
* Significantly lower at $P \leq 0.05$ using Student's t-test

cover was relatively low compared to graminoid cover, the frequency of some forbs was high. For example, kochia was the second most frequently encountered plant, many-flowered aster the fifth most and gumweed the seventh.

Shrub cover was almost entirely lacking on contaminated plots but comprised nearly 6% on uncontaminated plots. Prairie rose (*Rosa arkansana* Porter) and western snowberry (*Symphoricarpos occidentalis* Hook.) were significantly less frequent on contaminated plots but only the latter species had significantly less cover.

Uncontaminated plots had significantly higher percentage plant species cover and litter than contaminated plots (Table 3.4). At seven sites total plant species cover on contaminated plots was significantly lower than in uncontaminated ones, the average being about 43% on contaminated and 58% on uncontaminated plots. At all but three sites, litter cover was significantly higher in uncontaminated than contaminated plots: about 36% compared to only about 13%. The contaminated plots had significantly more bare ground than uncontaminated plots, about 44% compared to 6%, respectively.

Mean species richness was 14 species on uncontaminated plots and 13 species on contaminated plots. However, the mean Jaccard's similarity index was only 31.1%, indicating that species composition was different between contaminated and uncontaminated plots (Figure 3.3). Winter 2 similarity was exceptionally high with about 80% of the species common to both contaminated and uncontaminated plots.

Shannon's diversity was significantly lower at contaminated (0.45) compared to uncontaminated plots (0.63), indicating that, at the contaminated plot, one or a few species were dominant and the remainder rare. The Spatz's index (Figure 3.3), which illustrates how similar two communities are in species, was only 22.2%. Only Winter 2

Table 3.4. Percentage vegetation, litter cover and bare ground found on uncontaminated and contaminated plots at 14 field sites in southern Saskatchewan.

	Cover (%)					
	Vegetation		Litter		Bare Ground	
Site	U [a]	C [b]	U	C	U	C
Forget	62.27	54.79	36.15	6.48 *	1.58 ***	38.74
Manor 1	54.78	49.67	35.77	12.19	9.45 *	38.14
Manor 2	72.55	26.14 ***	26.14	5.87 ***	0.89 ***	67.99
Arcola 1	55.18	24.24 ***	42.11	12.09 ***	2.71 ***	63.68
Arcola 2	50.98	36.15 *	35.75	27.58	13.27 ***	36.27
Hassard	46.65	25.36 **	43.97	11.89 ***	9.38 ***	62.75
Winter 1	59.51	39.14 **	32.42	18.05	8.07 ***	42.81
Winter 2	56.53	56.13	37.78	12.39 **	5.69 ***	31.49
Winter 3	56.04	58.89	35.89	20.56 ***	8.07 **	20.55
Fosterton	49.93	38.96	36.89	29.30	13.18 *	31.74
Cantuar	50.43	39.03 *	47.36	0.00 ***	2.21 ***	60.97
Success 1	70.58	72.97	28.41	6.36 *	1.01 **	20.67
Success 2	65.61	28.92 ***	32.60	0.26 ***	1.79 ***	70.83
Success 3	56.39	51.18	38.22	12.72 ***	5.39 *	36.09
Mean	57.67	42.97 **	36.39	12.55 ***	5.91 ***	44.48

[a] U = Uncontaminated plot
[b] C = Contaminated plot
*, **, *** Significantly lower than adjacent plot at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively using Student's t-test

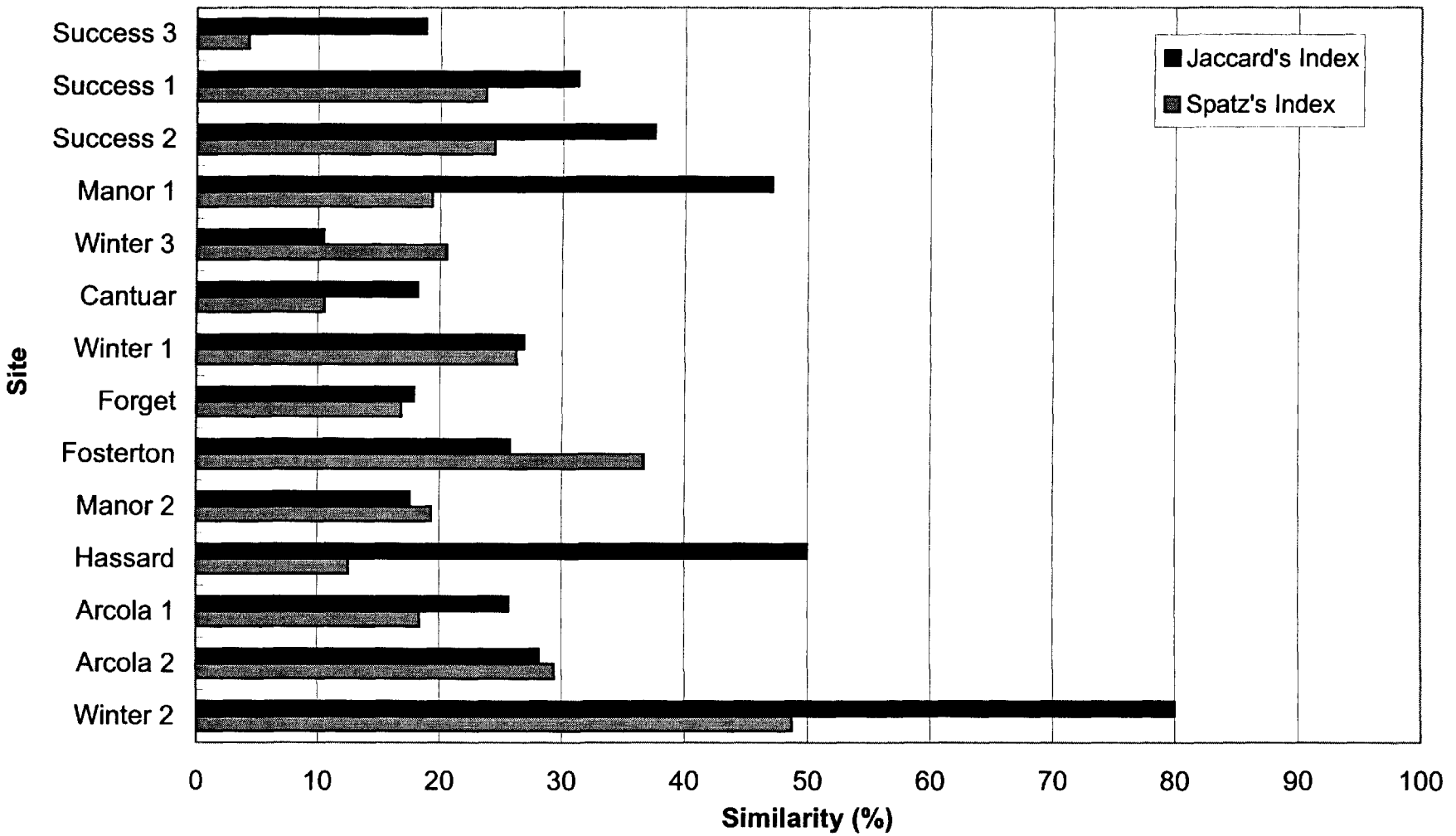

Figure 3.3. Jaccard's and Spatz's percentage similarity indices comparing contaminated to adjacent uncontaminated plots for each of 14 sites in Saskatchewan. Sites are sorted from most (top) to least (bottom) contaminated.

and Fosterton had Spatz's indices of greater than 30%. Although Hassard and Manor 1 had relatively high Jaccard's indices (i.e. 50% and 47%, respectively), their Spatz's indices were less than 20%, suggesting that species common to both plots did not always comprise a large percentage of cover.

The results of the CCA of contaminated and uncontaminated plots and environmental variables are shown in Table 3.5. Percent clay was positively correlated with axis 1. Percent silt and C:N ratio was negatively correlated and soil zone positively correlated with axis 2. Total petroleum hydrocarbons was weakly negatively correlated with axis 2. Available potassium was negatively correlated with axis 3. Plots high in clay were towards the right of the axis 1 vs. 2 ordination, those high in silt near the bottom and those high in sand near the left (Figure 3.4). In the axis 2 vs. 3 ordination silty soils were near the left (Figure 3.5). Plots in the black soil zone were near the top of the axis 1 vs. 2 ordination and the right of the axis 2 vs. 3 ordination. The brown soil zone was near the bottom of the axis 1 vs. 2 ordination and the left of the axis 2 vs. 3 ordination. Plots with high C:N ratios tended to be in the lower right of axis 1 vs. 2 ordination and near the left of the axis 2 vs. 3 ordination. In Figure 3.5 plots high in available potassium were near the bottom and those low in it near the top. Environmental variables with intraset correlations less than 0.45 were not shown on the ordination graph.

When functional attributes of plants on contaminated and uncontaminated plots were compared, differences were obvious. Most species found at the sites were in the grass (Poaceae) and aster (Asteraceae) families. Over 60% of the species and 80% of the vegetation cover were from plants in these two families on both contaminated and

Table 3.5. Intraset correlations on three axes for 14 variables using canonical correspondence analysis on uncontaminated and contaminated plots at 14 field sites in southern Saskatchewan.

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
Bare ground (%)	-0.361	-0.185	0.335
Litter cover (%)	-0.095	0.096	0.115
Silt (%)	0.169	-0.513	-0.255
Clay (%)	0.529	-0.178	0.236
EC (mS/cm)	0.170	-0.111	0.112
pH	-0.091	0.096	-0.001
Carbon (%)	0.068	0.211	0.013
Nitrogen (%)	0.012	0.292	-0.058
C:N	0.264	-0.452	0.262
Available Phosphorus (μg/g)	-0.129	-0.243	0.097
Available Potassium (μg/g)	0.028	0.040	-0.662
Time Since Initial Contamination	-0.240	-0.317	0.301
Soil Zone	0.070	0.820	-0.082
Total Petroleum Hydrocarbons (μg/g)	-0.007	-0.416	0.131

uncontaminated soil (Figure 3.6). However, while less than 40% of the species were in the grass family, they formed over 75% of the cover. The goosefoot (Chenopodiaceae) family was significantly more common in the contaminated plots as percentage of species but not vegetation cover. Non-native species were more common in

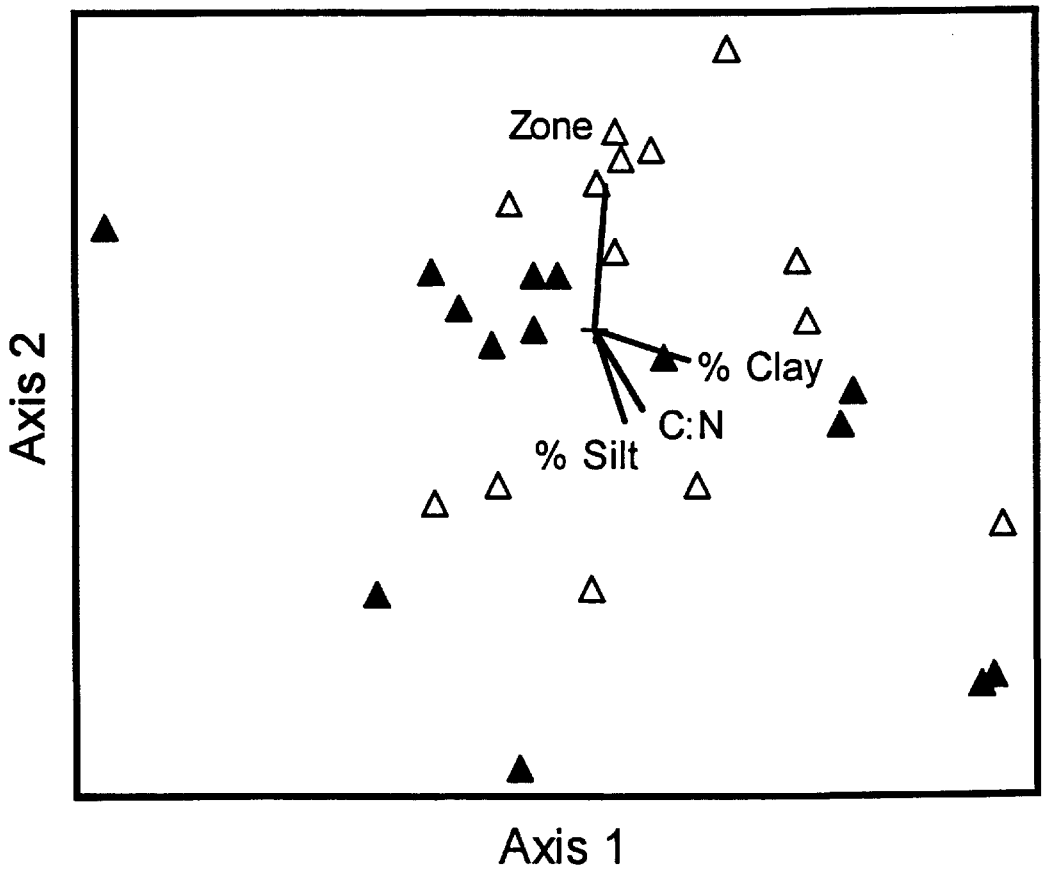

Figure 3.4. Canonical correspondence analysis diagram of axes 1 and 2 for contaminated (solid triangles) and uncontaminated (open triangles) plots and influential environmental variables (lines). The direction of the line indicates the variable gradient and its length strength of the intraset correlation. Where C:N = total carbon to total nitrogen ratio and zone = brown or black soil zone.

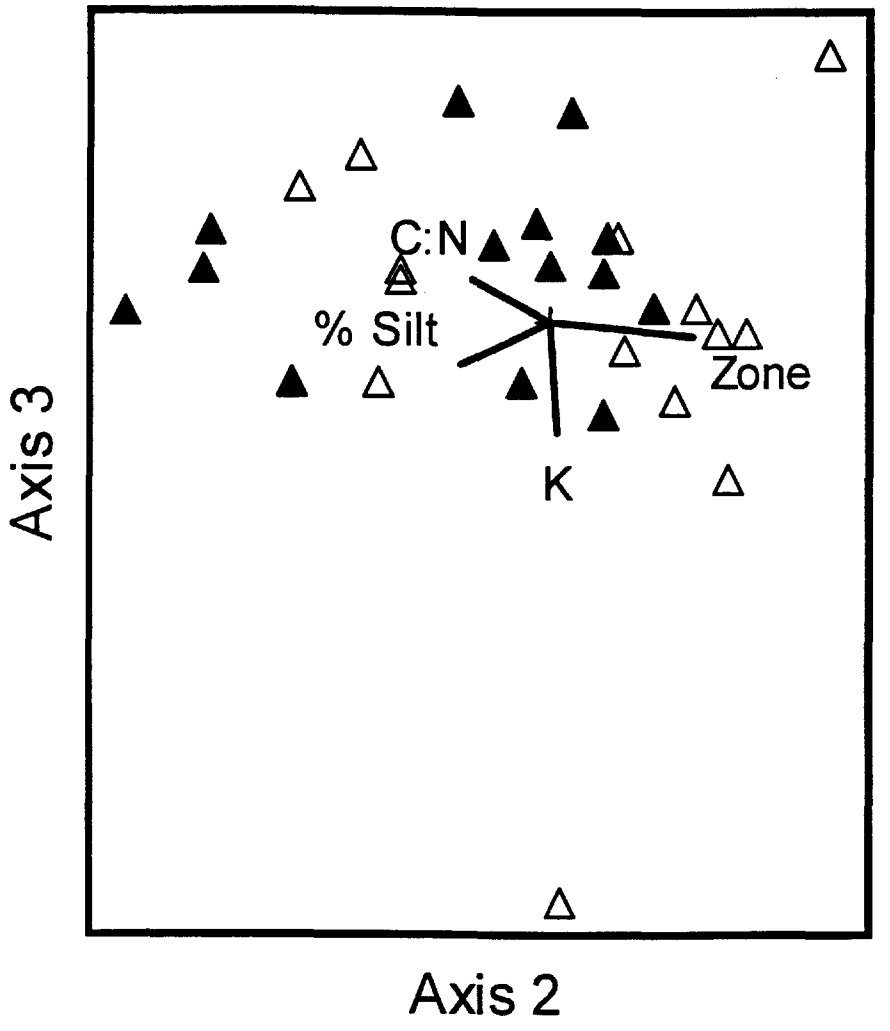

Figure 3.5. Canonical correspondence analysis diagram of axes 2 and 3 for contaminated (solid triangles) and uncontaminated (open triangles) plots and influential environmental variables (lines). The direction of the line indicates the gradient of the variable and its length the strength of the intraset correlation. Where C:N = total carbon to total nitrogen ratio and zone = brown or black soil zone.

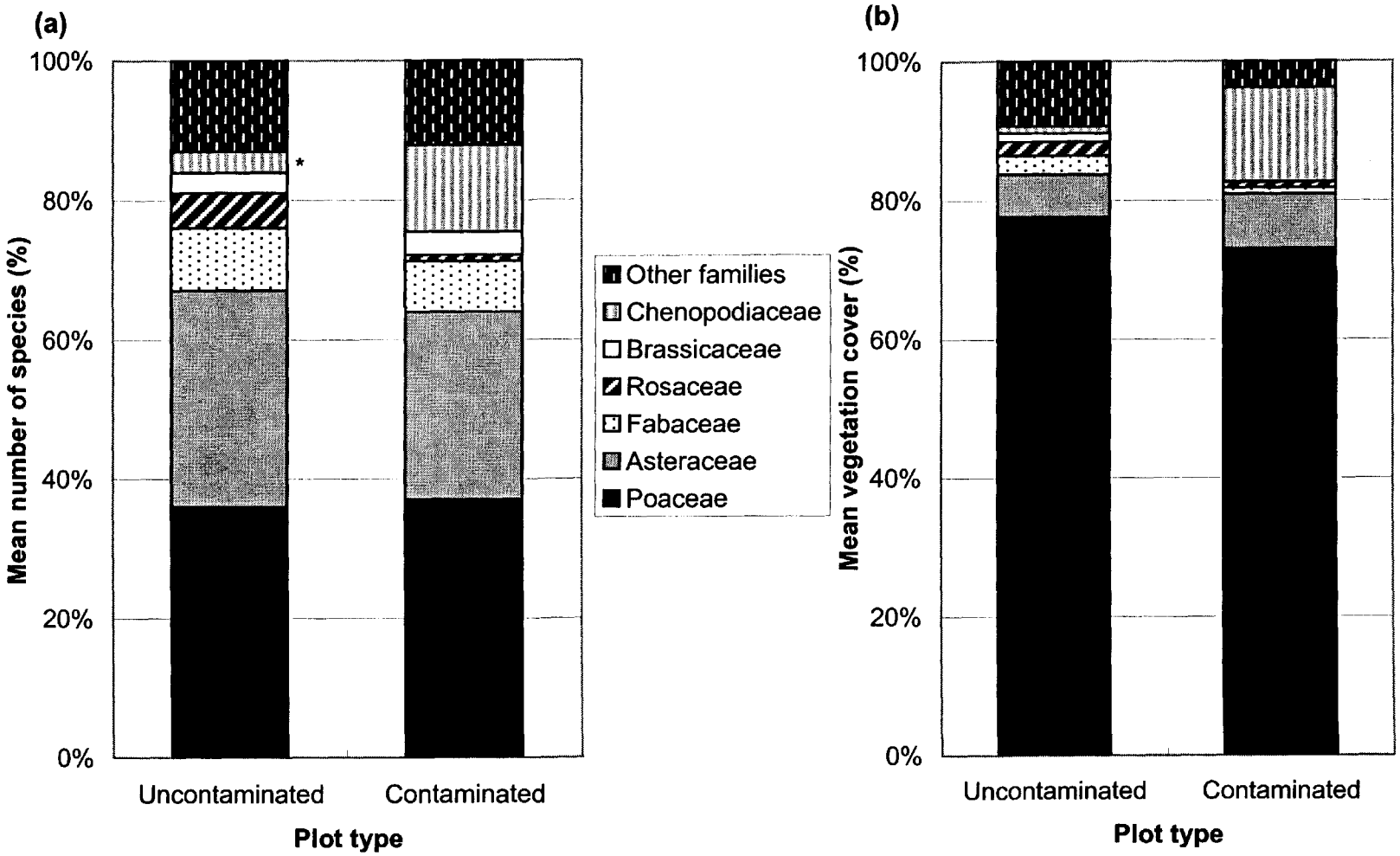

Figure 3.6. Percentage of (a) species and (b) vegetation cover at all 14 sites attributed to plants according to plant families. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

contaminated plots as percentage of species but not vegetation cover (Figure 3.7). About 5% of the species and 4% of the cover on uncontaminated plots were from plants capable of fixing nitrogen, but this functional group contributed only 3% of the species and less than 0.5% of cover on contaminated plots; these differences were not statistically significant (data not presented).

Species that are typically mycorrhizal comprised significantly more cover than non-mycorrhizal species on uncontaminated than contaminated plots (Figure 3.8). Unfortunately the typical mycorrhizal status of a large number of the species identified in this study is unknown. The contaminated plot with the lowest cover attributable to mycorrhizal species was the one that had been most recently disturbed, the Cantuar plot. Less than 0.05% of the cover on the contaminated plot at the Cantuar site was from typically mycorrhizal species in contrast to nearly 100% on the uncontaminated. In general, mycorrhizal species were more common at the plots with the longest time since initial contamination.

The number of woody and perennial species was significantly lower, and annuals and biennials significantly higher on contaminated compared to uncontaminated plots (Figure 3.9). Only woody species formed significantly less cover on contaminated plots. In both plots, perennials formed between 80-90% of the cover.

Although less than 40% of the species on uncontaminated plots could reproduce vegetatively, these species formed almost 67% of the vegetation cover (Figure 3.10). Plants reproducing vegetatively were significantly less common on contaminated plots in both number of species and percentage cover.

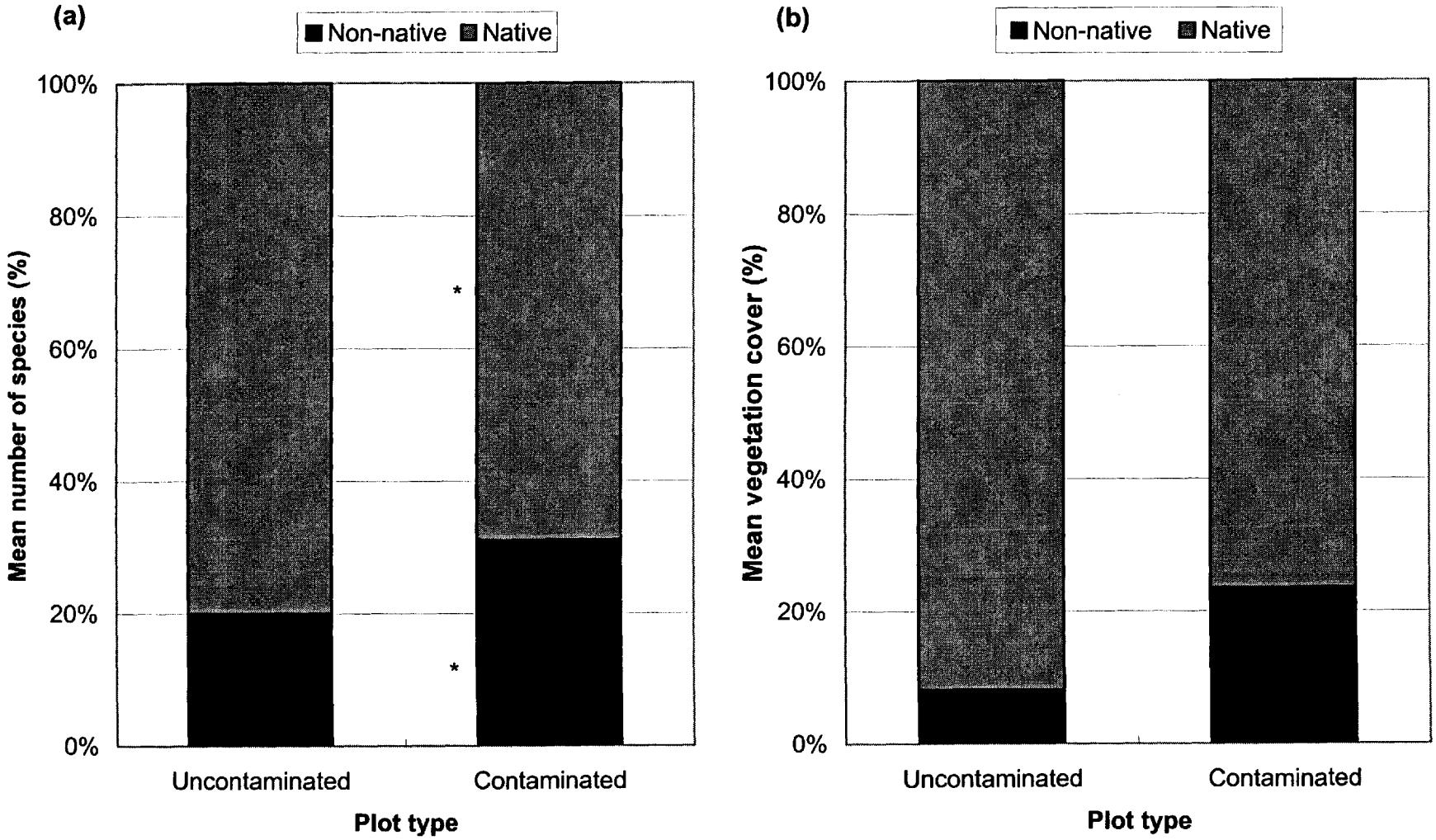

Figure 3.7. Percentage of (a) species and (b) vegetation cover at all 14 sites attributed to plants that are non-native or native to Canada. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

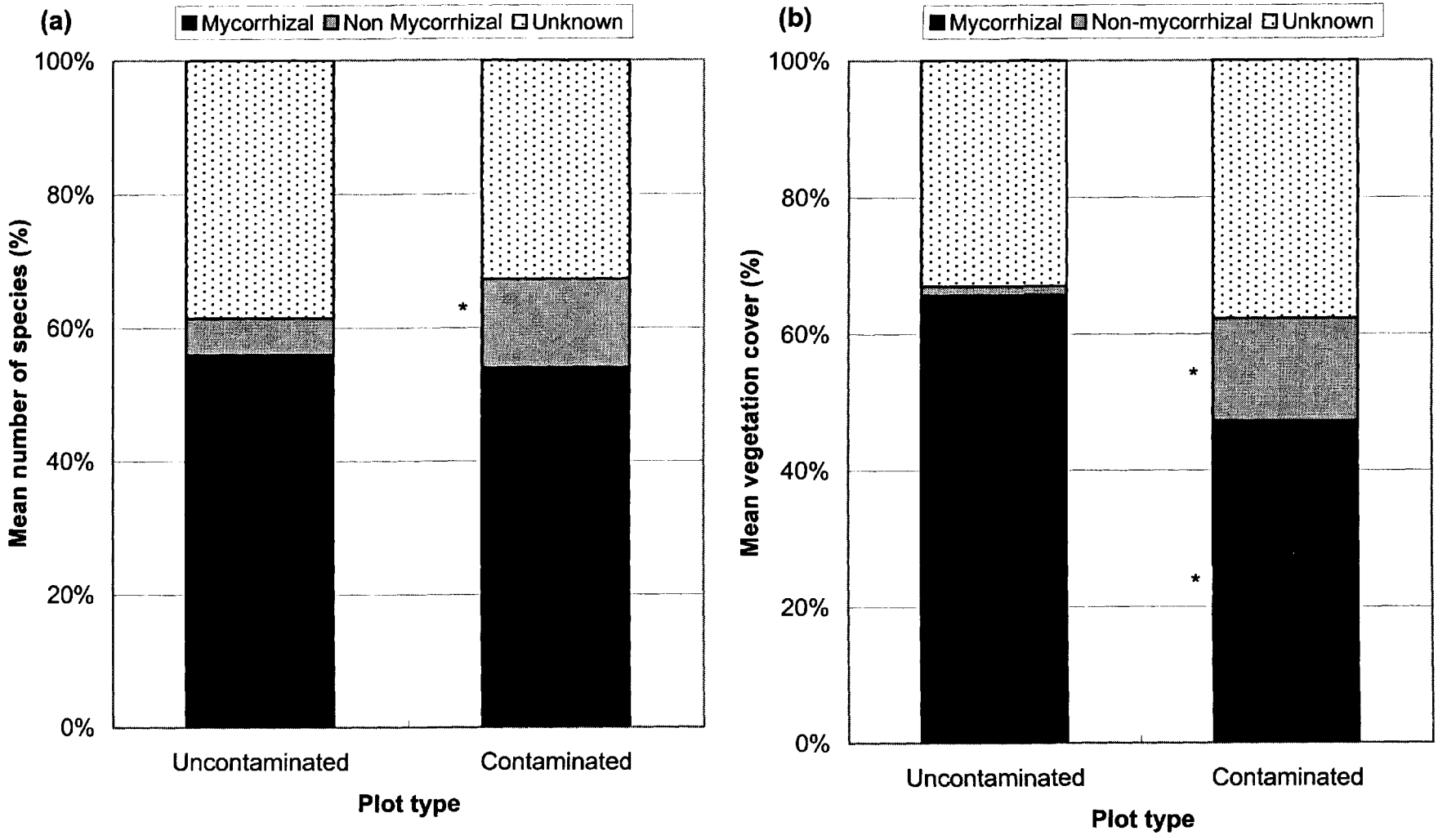

Figure 3.8. Mycorrhizae-forming status in terms of (a) mean percentage species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

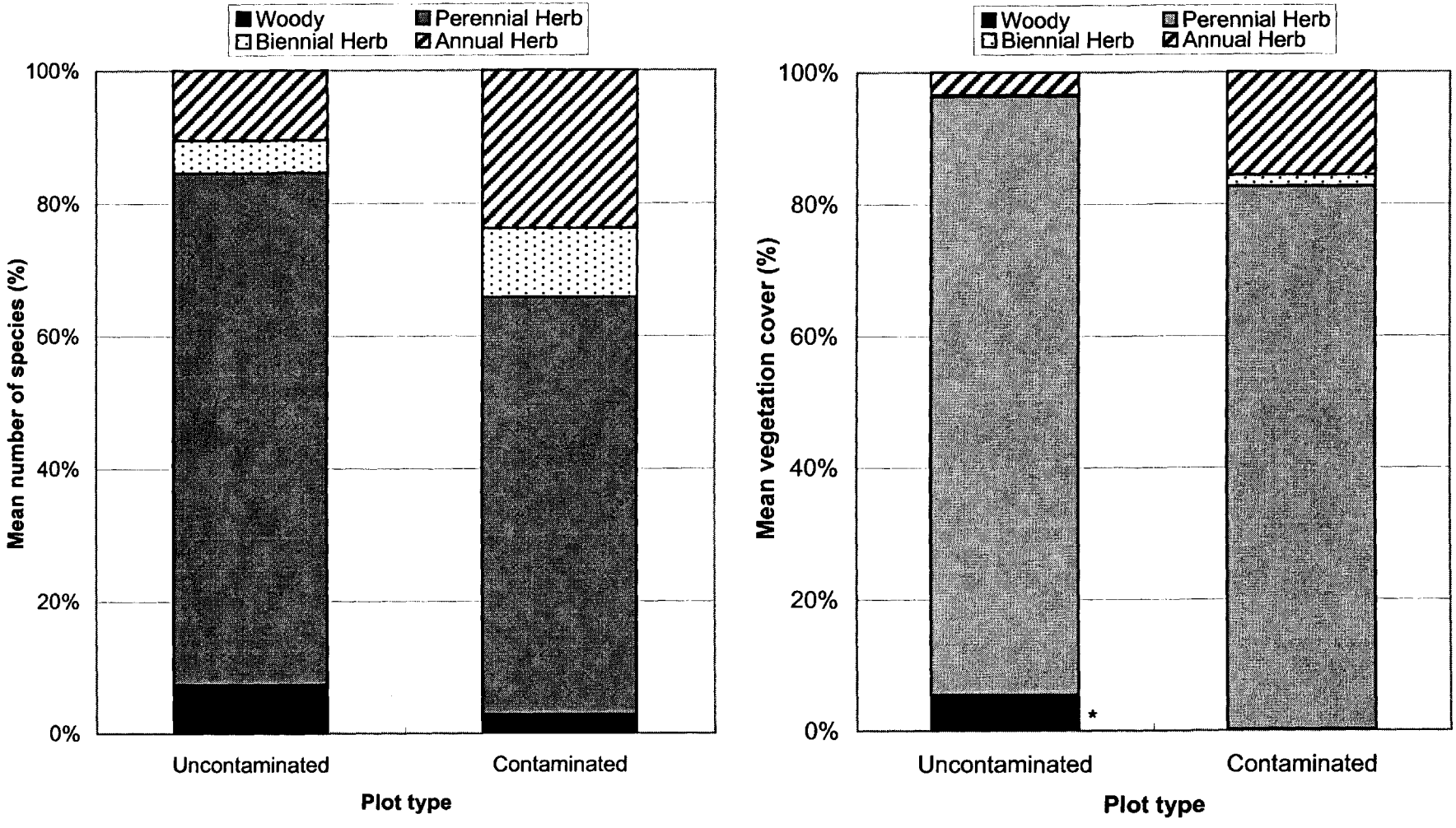

Figure 3.9. Life history status of all plants in terms of (a) mean percentage of species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

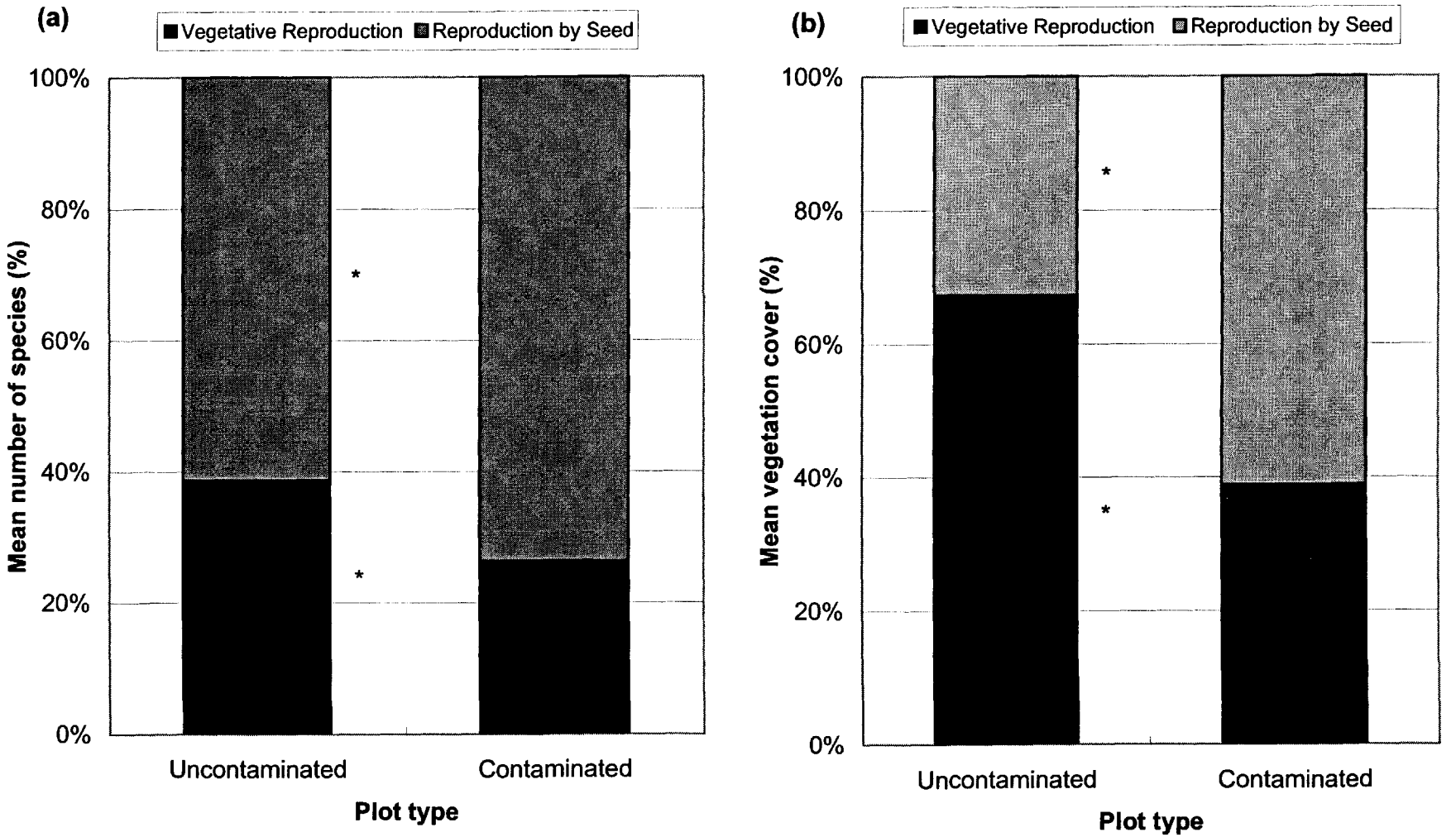

Figure 3.10. Reproductive mode of all plants in terms of (a) mean percentage of species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

Although wind-pollinated species comprised only about 40% of the species they formed almost 80% of the vegetation cover (Figure 3.11). The cover attributed to self-pollinating species was significantly higher on contaminated compared to uncontaminated plots but percentage of species was not.

Contaminated and uncontaminated plots had almost identical numbers of species in each dispersal category (Figure 3.12). However, species with unassisted and bird-dispersed seeds formed significantly less cover on contaminated plots.

Most plants had seed masses between 9.9 and 0.1 mg (Figure 3.13). Plants with seed masses between 9.9 and 1.0 mg were more common on contaminated soil and those with seeds less than 0.1 mg less common. Plants with seed masses between 9.9 and 1.0 mg formed significantly more cover on contaminated plots and those with masses between 0.9 and 0.1 mg less.

Excluding the three contaminated plots lacking nearby native prairie did not affect which species were most abundant on contaminated plots (data not presented). Diversity of the contaminated plots increased to 0.51 but was still significantly lower than the uncontaminated plots. The percentage of species with seed masses between 9.9 and 1.0 mg were significantly more common at $P \leq 0.05$ on contaminated soil. The cover attributed to species that are typically mycorrhizal, reproduce vegetatively and have unassisted seeds was no longer significantly different between contaminated and uncontaminated plots (data not presented).

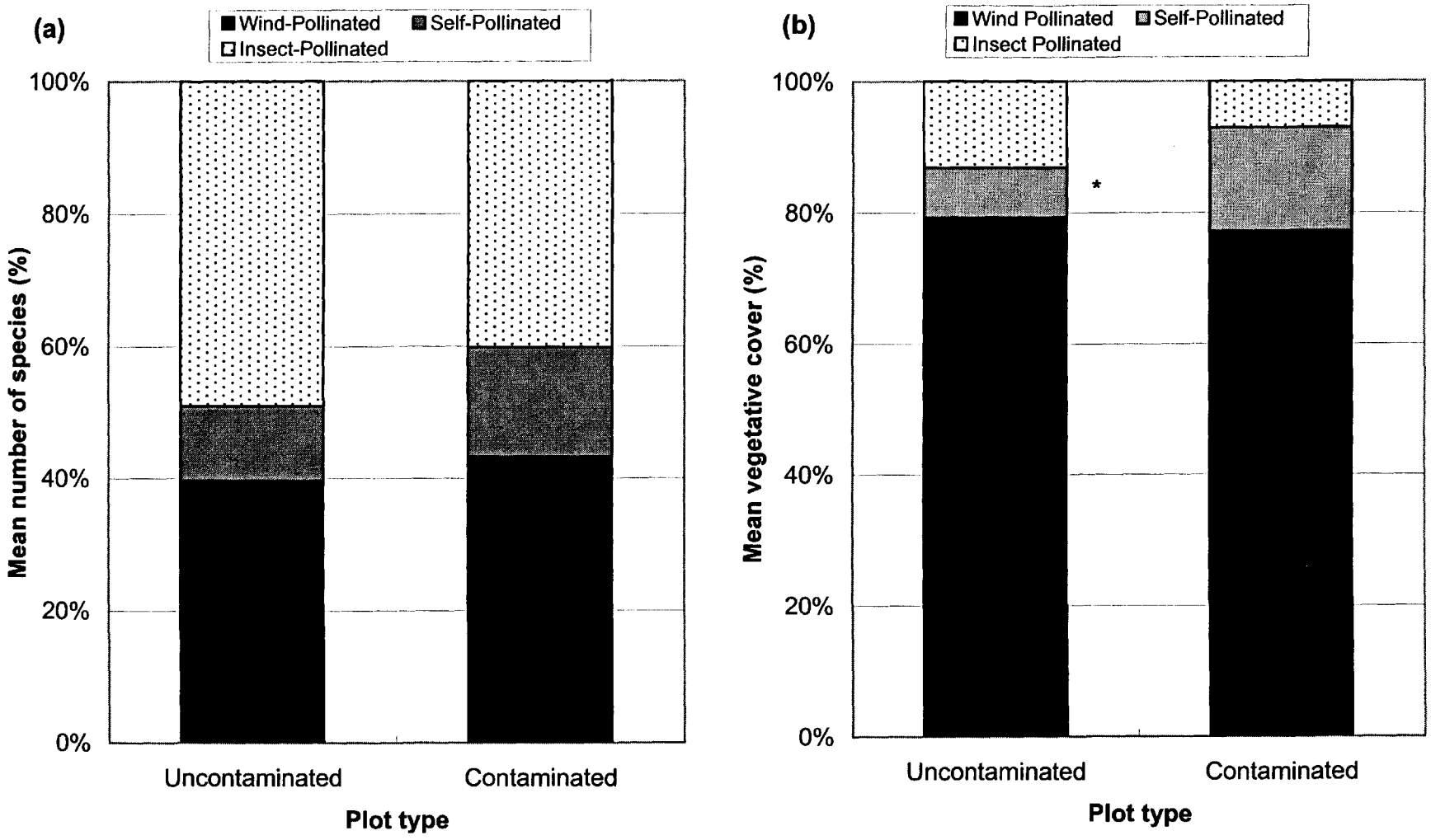

Figure 3.11. Pollination mode of all plants in terms of (a) mean percentage of species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

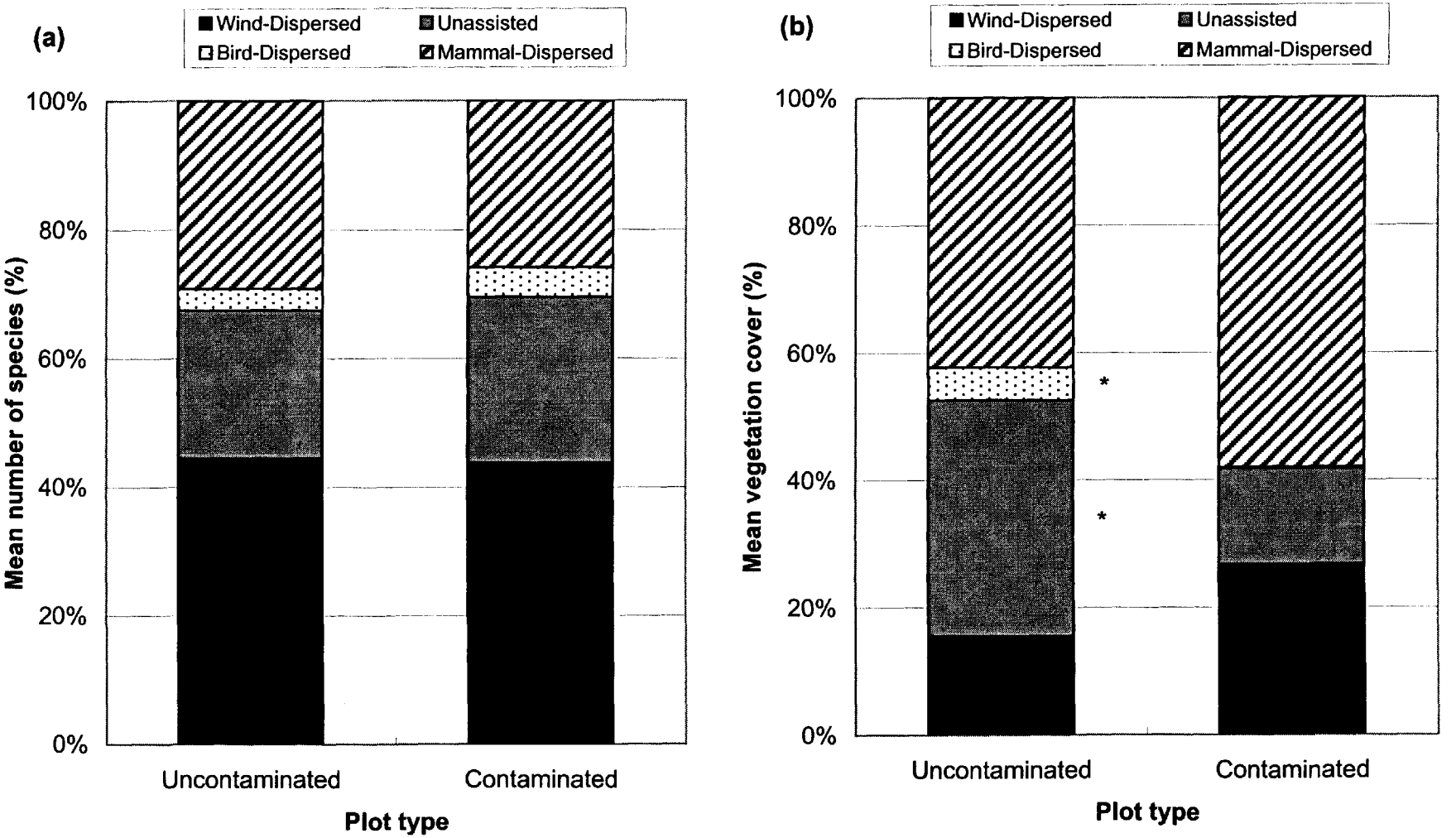

Figure 3.12. Seed dispersal mode of all plants in terms of (a) mean percentage of species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

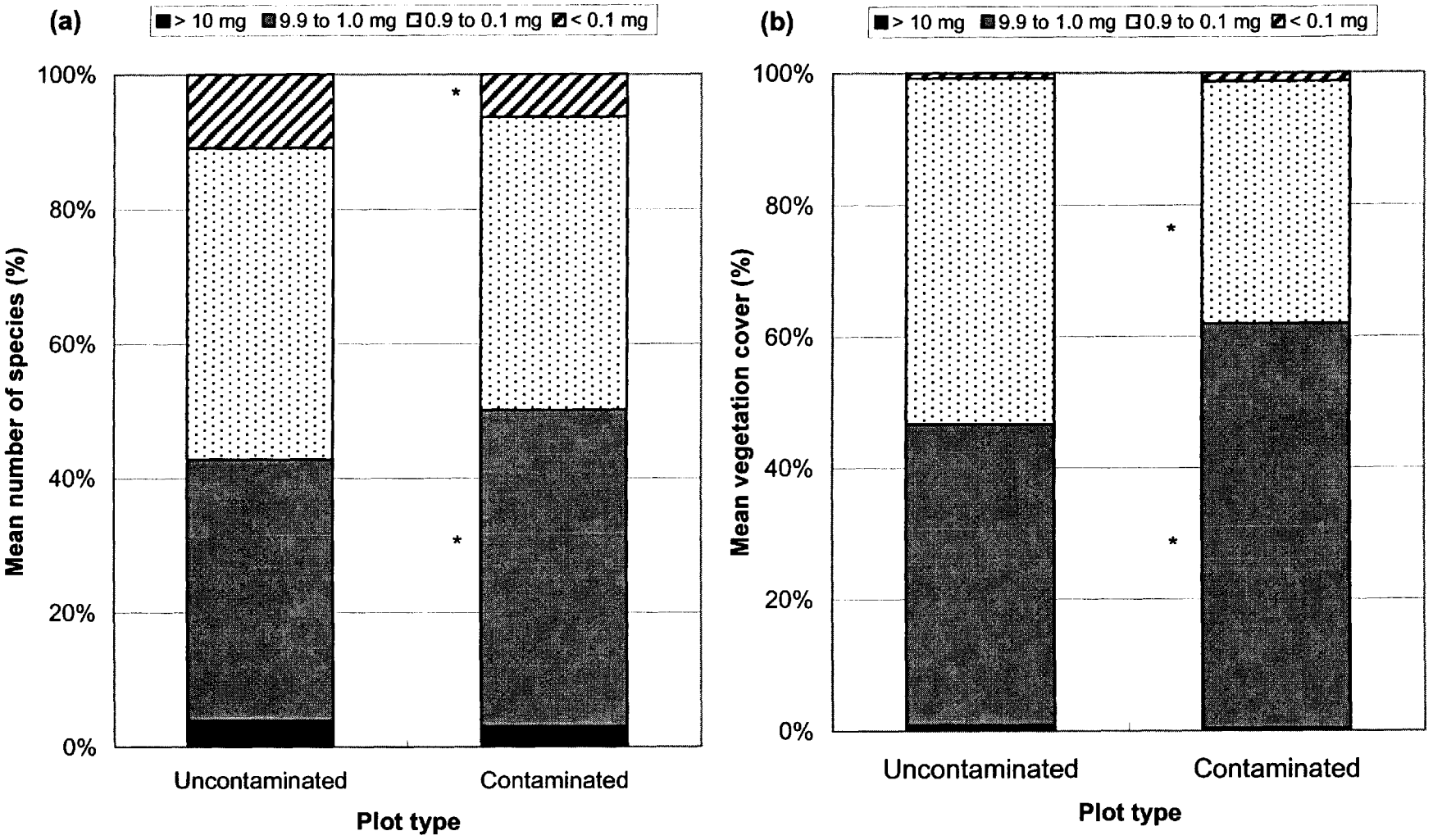

Figure 3.13. Seed size class of plants in terms of (a) mean percentage of species and (b) mean percentage vegetation cover at 14 sites. Significant differences ($P \leq 0.05$) in values between uncontaminated and contaminated plots denoted by *.

3.5 Discussion

Although species richness was not significantly different between contaminated and uncontaminated plots, diversity was significantly lower on contaminated soils. The species most responsible for low diversity were salt grass, which was less common on uncontaminated plots, and Kentucky bluegrass, hairy golden aster and western snowberry, which were less common on contaminated plots. Similarity was low because the frequency and abundance of smooth brome, Kentucky bluegrass, kochia, prairie rose and western snowberry were significantly different between contaminated and uncontaminated plots. Differences in the frequency and abundance of less common graminoids, forbs and shrubs also affected plot similarity. Species composition and abundance is different between contaminated and uncontaminated plots due to variation in species' abilities to tolerate crude oil (Racine, 1994), soil disturbance (Grime, 1979; Wali, 1999) and low fertility (Wilson and Tilman, 1991). The contaminated plots are at an early stage of ecological succession as indicated by the abundance of ruderals (e.g. annuals, biennials, herbs) (Grime, 1979; Denslow, 1980). Many of the species common on contaminated plots also colonize coal mine spoil (e.g. kochia, wild barley, gumweed, many-flowered aster) (Wali, 1999).

There were some functional groups that were more common on contaminated soils. Species that were typically non-mycorrhizal were more common on contaminated than uncontaminated plots, supporting the hypothesis of Reeves and associates (1979). One plant family that is typically non-mycorrhizal, the goosefoot family (Chenopodiaceae), was more common on contaminated than uncontaminated plots. This is because both soil disturbance (Miller, 1978; Jasper et al., 1989) and the presence

of hydrocarbons (Cabello, 1997; Leyval and Binet, 1998) reduce mycorrhizal infectivity. As plant roots from the field sites were not examined for mycorrhiza, it is possible that some of the cover attributed to mycorrhizal plants should not have been. Several species mycorrhizal on undisturbed land do not form mycorrhiza on disturbed land, including western wheatgrass (Miller, 1978) and slender wheatgrass (Zak and Parkinson, 1982). If western and slender wheatgrass were not mycorrhizal on the contaminated plots examined in this study, the percentage cover due to non-mycorrhizal species would increase from about 13% to 22%. Thus some of the vegetation cover on contaminated land may have been from typically mycorrhizal species that had not actually formed mycorrhiza. Another factor that must be considered is that species differ with regards to their mycorrhizal dependence; some plants cannot even germinate without coming in contact with mycorrhiza while others are facultative mycotrophs (Brundrett, 1991). Mycorrhizal dependence is higher in C4 grasses than C3 grasses, possibly because C4 grasses grow during the drier part of the year and need mycorrhiza to supply them with water (Betivenga and Heterick, 1992). Interestingly, the only C4 plants that were common on contaminated plots were non-mycorrhizal ones (i.e. kochia, Russian thistle). The dominant grasses on contaminated plots were all C3 species. Thus mycorrhizal dependence of a plant also affects colonization of contaminated soil.

Woody species were less common on the contaminated sites as predicted by Hobbs et al. (1988) and Johnson (1981). However, woody species *per se* are not necessarily sensitive to hydrocarbons because several studies have found woody species to be abundant on hydrocarbon-contaminated soils (Mackey and DePuit, 1985; Olson and Fletcher, 2000). Prairie rose and western snowberry, the two most common shrubs

on the uncontaminated plots, may be hydrocarbon-sensitive, given their lower frequency on contaminated plots. Another factor affecting the abundance of woody species is that their seeds are less likely to arrive at disturbed sites, as they are typically bird-dispersed. Birds are less likely to visit contaminated sites due to the low plant cover (Prach and Pyšek, 1999).

Plants with unassisted seeds were less frequent on contaminated plots as predicted by Johnson (1981) and Prach et al. (1997), due to lower mobility (Prach and Pyšek, 1999). Heavier-seeded species were more common on contaminated than uncontaminated plots, suggesting that they may indeed be more tolerant of poor soil conditions than small-seeded species (Poorter and Garnier, 1999).

Self-pollinated species were more common on contaminated plots because they are more likely to be reproductively successful on disturbed sites with low plant cover than species requiring insects for pollination (Johnson, 1981). Perennials that reproduce vegetatively were less common on contaminated soil because they produce fewer seeds than annuals, and are less likely to colonize a disturbed area (Smith et al., 1997). Non-native species were more common on the contaminated plots likely because the soil disturbance that accompanied contamination provided a niche for such species.

Nitrogen-fixing species are predicted to be more common on hydrocarbon-contaminated (Gudin and Syratt, 1975) and nitrogen-poor soils (Stewart, 1967; Harper, 1977). However, this hypothesis was not supported by the results of this study: nitrogen-fixing plants were present in similar abundance on hydrocarbon-contaminated and uncontaminated plots. The ability of indigenous nitrogen-fixing bacteria to tolerate hydrocarbons is unknown; a low abundance of these bacteria may limit growth of their

symbionts in contaminated soils. Hydrocarbons may also affect nodulation ability. Suominen et al. (2000) found that *R. galegae* could survive in m-toluate soil with concentrations as high as 3,000 ppm, but that the ability to nodulate *Galega orientalis* decreased. Nitrogen-fixing bacteria also have different levels of tolerance to arid (Mohammad and Johnson, 1997) and saline (Elsheikh-Elsiddig, 1998) conditions, which occur at many of the sites. Most native legumes in Saskatchewan are mycorrhizal (Currah and Van Dyk, 1986); since mycorrhizae are less common in contaminated and disturbed soil (Stahl and Williams, 1986), legume germination and growth may be negatively affected. Fenner and Lee (1989) note that legumes require more nitrogen for initial growth than is present in their seeds due to the high nitrogen demands of nodule formation. Low nitrogen availability may also explain why legumes were rare in the hydrocarbon-contaminated sites visited.

It is hypothesized that succession will be more rapid if late successional species grow near a disturbed site (Denslow, 1980). This research supports this hypothesis as diversity of the contaminated plots increased slightly with the plot exclusions, meaning that the presence of nearby native prairie affected the abundance of some species. Further, species that reproduced vegetatively, had unassisted seeds or were typically mycorrhizal were not significantly different between contaminated and uncontaminated plots when those plots without adjacent native prairie were excluded from the analyses. This means species in these functional groups are less able to colonize isolated patches of land than species in other groups. When surveying contaminated sites for hydrocarbon-tolerant species, distance to native plant communities should be noted, as it may affect species composition and abundance.

The soils at contaminated plots had higher carbon to nitrogen ratios and pHs, and lower total nitrogen and available phosphorus. The lower nitrogen is due to soil disturbance, as hydrocarbon addition increases total nitrogen slightly (Stahl and Williams, 1986). The high carbon to nitrogen ratio is also primarily due to soil disturbance as total carbon was not significantly different between contaminated and uncontaminated plots. Since both soil disturbance and hydrocarbon addition may increase pH (Udo and Fayemi, 1975) and decrease available phosphorus (Naeth et al., 1987; Rowell and Florence, 1993), which factor is having a greater effect cannot be determined. At some contaminated plots low potassium and total carbon and high EC may have negatively affected plant growth. For example, at Manor 2 EC was the soil variable that likely affected plant productivity because total nitrogen, available phosphorus and C:N ratio were not significantly different. Other plots with extremely high EC (i.e. >5 mS/cm) include the contaminated plots at Cantuar, Manor 1 and Success 2 and the uncontaminated plots at Manor 1 and 2 and Success 1. The low fertility and adverse soil chemistry at the contaminated plots decreased vegetation and litter cover.

The environmental variables that most influenced the CCA were soil zone, soil texture (i.e. % clay and % silt), available potassium and C:N ratio. However the correlations were not very strong (i.e. less than 0.67 for all variables except soil zone). This can be explained by the large amount of variability in several soil characteristics among the various sites. Some plots were very high in sand (e.g. Winter 2 and Manor 1 had over 80% sand) while others were high in clay (e.g. Success 1 had greater than 40% clay). Carbon to nitrogen ratio ranged from 11:1 at an uncontaminated plot and 51:1 at

a contaminated one. Potassium was over 800 μg/g at some plots and less than 200 μg/g at others. Total petroleum hydrocarbon concentration was weakly negatively correlated with axis 2 suggesting that it was somewhat influential on the plant community. However, contaminated plots were not clustered together on the ordination, suggesting that species composition was not similar. This is likely because species composition was strongly affected by soil zone and texture. Plant communities at matched uncontaminated and contaminated plots were not always close together, due to differences in C:N ratio, available potassium and disturbance. EC was very weakly correlated with axis 1, meaning that this variable was not influencing plant communities as much as other variables.

A low Jaccard's index means that either fewer species were found on contaminated than uncontaminated plots or that species composition between paired plots was different. Low Jaccard's indices at Forget, Fosterton and Winter 3 was due, at least in part, to the smaller number of species found on the contaminated plots. However, species richness between the plots was similar; a difference in species composition was the primary factor causing low Jaccard's indices. This means that species present on uncontaminated plots did not always occur on contaminated plots and visa versa. Sites with the highest Jaccard's index (i.e. Winter 2 and Hassard) were completely surrounded by diverse native prairie, which likely increased the number and variety of seeds arriving. When species abundance was taken into account using Spatz's index, similarity decreased at most sites. A low Spatz's index but a high Jaccard's index (e.g. Hassard, Manor 1, etc.) indicates that dominant species at the two plots were different due to large differences in soil properties (i.e. C:N ratio, EC, etc.).

A high Spatz's index but a low Jaccard's index (i.e. Arcola 2, Fosterton and Winter 3) indicates that the dominant species were similar at the two plots.

Of all the species found on contaminated plots western and northern wheatgrass are the most promising for reclamation. These two species are native, not invasive and readily available from seed suppliers. On contaminated soils that are saline, the native grasses, salt grass and Canby bluegrass, would be more appropriate as they are salt tolerant. Although kochia and wild barley were most common species on contaminated plots, they are less desirable than native grasses because they are common weeds on cultivated land. The native forbs, many-flowered aster and gumweed, although comprising little cover, had high frequencies on contaminated plots, suggesting they are also hydrocarbon tolerant. The roots of all these plant species were observed growing through hydrocarbon clods, supporting the assertion that they are indeed hydrocarbon tolerant, not just tolerant of salts. These eight species should be examined for their ability to accelerate hydrocarbon degradation, as it may explain their tolerance.

3.6 Conclusions

Five grasses and three forbs tolerant of hydrocarbon-contaminated soils were identified. Similarity of contaminated to uncontaminated plots was low due to differences in species composition and abundance. Although species in certain functional groups were less common on contaminated soil (i.e. unassisted seed dispersal, vegetative reproduction) this may be due to poor dispersal capabilities rather than hydrocarbon intolerance. Plants with large seeds were more common on contaminated than uncontaminated soil, supporting the hypothesis that large-seeded

species have an advantage on contaminated sites. When native prairie was nearby, diversity and functional groups were more similar between contaminated and uncontaminated plots. The low vegetation cover and litter of contaminated plots are likely due to high pH and carbon to nitrogen ratios, and low nitrogen and phosphorus resulting from hydrocarbon contamination and soil disturbance. Soil zone, texture, C:N ratio and potassium availability were the environmental variables most influencing the similarity of the plant communities. Had the sites been more similar in their location and soil texture total petroleum hydrocarbons would likely have accounted for more of the variability in the plant communities.

4.0 ABILITY OF COLD-TOLERANT SPECIES TO GROW IN PETROLEUM HYDROCARBON CONTAMINATED SOIL

4.1 Abstract

Phytoremediation of hydrocarbons in soil involves plants and their associated microorganisms. Differences in environmental conditions and restrictions on species importation mean that each country may need to identify indigenous plants to use for phytoremediation, particularly in native ecosystems where the use of non-native species is restricted. Screening plants for hydrocarbon tolerance before screening for degradation ability may prove more economical than screening directly for degradation. Thirty-nine plants native or non-native but adapted to conditions in western Canada were assessed for their ability to survive under growth chamber conditions in crude oil-contaminated soil.

Crested wheatgrass (*Agropyron pectiniforme* R. & S.), smooth brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), alfalfa (*Medicago sativa* L.), yellow sweet-clover (*Melilotus officinalis* (L.) Lam.), white clover (*Trifolium repens* L.), pasture sage (*Artemisia frigida* Willd.), prairie cinquefoil (*Potentilla pensylvanica* L.), fringed brome (*Bromus ciliatus* L.), wild licorice (*Glycyrrhiza lepidota* (Nutt.) Pursh) and Indian breadroot (*Psoralea esculenta* Pursh) exhibited phytoremediation potential based on survival. We determined the effect of increasing crude oil concentrations on total and root biomass, and relative

growth rate of those species with the highest survival. The addition of 5000, 10,000 and 50,000 ppm (wt/wt) crude oil to soil significantly decreased total biomass and relative growth rate of all species except Indian breadroot. Root biomass significantly decreased with crude oil addition in all species except Indian breadroot and prairie sage. Total biomass production in contaminated soil had a significant negative correlation with the relative growth rate in uncontaminated soil.

4.2 Introduction

Phytoremediation, the use of plants and their associated microorganisms to degrade or contain soil contaminants, may be an efficient biotechnology for the treatment of hydrocarbon-contaminated soil (Reilley et al., 1996). Plants improve degradation by increasing microbial biomass in the rhizosphere (Crowley, 1997), and releasing cometabolites (Haby and Crowley, 1996) or degradation-accelerating enzymes (Gunther et al., 1996). Advantages of phytoremediation include lower costs than engineering techniques and public support (Germida et al., 2002). Phytoremediation would be ideal in sensitive, remote natural areas like sand dunes because soil disturbance and site maintenance is low. Although some plants have been identified as hydrocarbon phytoremediators (Phytoremediation Research Team, 2001), they may not be suitable in all parts of the world. Most countries are reluctant to import non-native species due to ecological problems that may occur when they are introduced. Furthermore, non-native plants may be unable to tolerate the soil and climate unique to every country. A screening protocol is needed to help scientists identify plants capable of degrading hydrocarbons.

Several protocols have already been suggested to screen plants for hydrocarbon degradation ability. Liste and Alexander (1999) suggest a colorimetric method to screen plants for phenol production as a way of identifying phytoremediators. Plants with higher phenol production degrade more phenanthrene than those producing less (Liste and Alexander, 1999). However, this method does not test the ability of plants to germinate in contaminated soil, a concern if seeds rather than seedlings will be used for revegetation. Kulakow and associates (2000) screened 29 species of grasses and legumes for survival and degradation on weathered, petroleum hydrocarbon-contaminated soil. They selected candidate plants based on a literature search, grew them on contaminated field soil for 60 or 180 days and determined biomass production and degradation rate. However, the high cost of hydrocarbon analysis may make screening large numbers of plants for degradation prohibitive. Reducing the number of candidate species for degradation testing by first screening plants for their ability to tolerate hydrocarbons would be more cost-effective.

One way to screen for hydrocarbon tolerant species may be to determine the relative growth rate (RGR) of plants. It is well known that plants with low RGR tend to be more common on infertile soils (Lambers and Poorter, 1992). Furthermore, stress-resistance and RGR are physiologically linked (Chapin et al., 1993; Weinstein and Yanai, 1994). Elias and Chadwick (1979) hypothesize that plants with low RGR will be more successful for reclamation of infertile, disturbed lands, such as abandoned mines, than those species with high RGR, because the former require fewer nutrients and water. They suggest growing candidate plants under ideal conditions to determine the maximum RGR (if it is not listed in existing literature) and selecting species with low

RGR for reclamation (Elias and Chadwick, 1979). Hydrocarbon-contaminated soils often have low fertility due to high C:N and C:P ratios (Xu and Johnson, 1995). Furthermore, hydrophobicity of contaminated soils inhibits water uptake by plants, creating more arid growth conditions (Chaîneau et al., 1997). However, to date no studies have tested whether plants with low RGR perform well on hydrocarbon-contaminated soil.

Field soil contaminated with 18,000 ppm oil refinery waste was used by Kulakow and associates (2000) to screen plants for degradation but this may be inappropriate for tolerance screening. Obtaining a control soil for comparison to a contaminated field soil can be difficult, especially if it is also contaminated with brine water or heavy metals, or was subject to herbicide or soil sterilant applications. Species identified as hydrocarbon tolerant may actually be tolerant of other soil contaminants, particularly if the concentration of the hydrocarbon is low. Some plants may be tolerant of small quantities of hydrocarbons, but not large quantities. Yield reduction in corn (*Zea mays* L.) is only 30% in soil contaminated with 11,000 ppm (wt/wt) crude oil but over 90% in soil with crude oil concentrations greater than 40,000 ppm (wt/wt) (Udo and Fayemi, 1975). Using only one contaminated field soil to screen plants may prevent identification of partially tolerant species. By using soils to which hydrocarbons have been added for screening experiments, the effect of only the hydrocarbon on plant growth can be observed. Furthermore, the concentration of hydrocarbons is easily manipulated.

The objective of this study was to screen species found in the northern Mixed Grass Prairie for hydrocarbon tolerance. The first screening examined the ability of

selected plants to emerge and survive in one field- and three artificially-contaminated soils for five weeks. Twelve species with the highest survival were further screened for total and root biomass production and RGR. The relationship between RGR of the plants in uncontaminated soil and total biomass in contaminated soils was investigated.

4.3 Materials and Methods

4.3.1 Survival screening

Native species germinate optimally in different types of soil (Evans et al., 1977) so three commercially available, artificial organic soils (i.e. soils 1, 2 and 3) with different chemical and physical properties (Table 4.1) were selected. All soils were analyzed for pH (McLean, 1982) and electrical conductivity (EC) (Rhoades, 1982) using a 1:2 soil to solution ratio, and percentage organic matter (% OM) by loss on ignition (Nelson and Sommers, 1982) (Envirotest Laboratories, Saskatoon, SK). Available nitrate (NO_3-N) was extracted from the soil using a dilute (0.001 N) calcium chloride solution (Martin, 1993) (Envirotest Laboratories, Saskatoon, SK). Nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye was measured colorimetrically at 520nm. Available orthophosphate P was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994) (Envirotest Laboratories, Saskatoon, SK). The orthophosphate ion reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form

a complex. This complex was reduced with ascorbic acid to form a blue complex, which was measured colorimetrically by auto analysis at 880 nm. Available potassium was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994) (Envirotest Laboratories, Saskatoon, SK). The extract was mixed with lithium nitrate, nitric acid and lanthanum oxide as an internal standard and passed into the burner of a flame photometer. Intensity of light emitted was measured at 768 nm. After organic matter oxidation with 1N H_2O_2 soil texture was determined using the pipette method (Gee and Bauder, 1986).

Table 4.1. Selected characteristics of soils used to screen for hydrocarbon tolerance in 39 species native or non-native in southern Saskatchewan.

	Soil Texture	OM [a] (%)	pH	EC (mS/cm)	NO_3-N (μg/g)	P (μg/g)	K (μg/g)
Soil 1	Artificial Organic	78	5.9	0.47	230	8.8	67
Soil 2	Artificial Organic	31	7.4	0.54	90	21.0	180
Soil 3	Artificial Organic	18	6.9	0.33	23	5.5	65
Field U[b]	Sandy Loam	4	6.8	0.24	11	19.0	340
Field C[c]	Sandy Loam	2	9.0	0.45	3	2.0	87

[a] Organic matter
[b] Uncontaminated Chernozemic reference soil
[c] Chernozemic soil contaminated with 20000 crude oil (wt/oven-dry wt) 5 years ago

Soils 1-3 were passed through a 5-mm screen to break up clods of soil and artificially contaminated with crude oil (Imperial Oil, Calgary, AB) in amounts equaling 1,000 (0.1%), 5,000 (0.5%), 10,000 (1%) and 50,000 (5%) ppm. The soil/oil mixture

was shaken vigorously for two minutes in a sealed plastic storage container to ensure even distribution. Chernozemic soil from a crude oil spill that occurred five years ago in southern Alberta (wt/oven-dry soil wt) and an uncontaminated reference soil from southern Saskatchewan with a similar texture, were obtained.

The plant species tested were chosen because they are commonly available for reclamation in western Canada (Jorgenson, 1997). Seeds of seven non-native species were obtained from a local supplier (Early's Farm and Garden Centre, Saskatoon); the seeds were from southern Saskatchewan seed growers. Seeds from 32 native species including 11 grasses, seven legumes and 14 forbs were obtained from local growers that harvest wild seed from within 50 km of their farms and grow it on their land (Prairie Mountain Seeds, Arcola; Blazing Star Wildflower Seed Co., St. Benedict; and the Mixed Grass Prairie Habitat Restoration Project, Last Mountain Lake, SK). The seeds were between nine and 12 months old when used in the experiments and had been air dried after harvest and stored at 5 °C.

The seed lots were examined to remove empty caryopses, awns, glumes and seeds that were damaged or unusually small. Seed treatments were applied when recommended in the literature (Currah et al., 1983; Young and Young, 1986; Nernberg, 1994; Steffen, 1997; Pahl and Smreciu, 1999) (Table 4.2). Scarification of legumes consisted of rubbing the seeds between two sheets of sandpaper for approximately one minute. Acid scarification of grasses consisted of dipping the seeds into 1N hydrochloric acid for 8-10 minutes. Stratification consisted of placing the seeds on moistened filter paper in petri dishes and placing them in a dark refrigerator at 5 °C for

Table 4.2. Scientific and common names of native species screened to which seed treatments were applied.

Scientific Name	Common Name	Seed Treatment
Native Forbs		
Achillea millefolium L.	Common yarrow	Stratification[1]
Artemisia frigida Willd.	Pasture sage	Stratification
Aster ciliolatus Lindl.	Lindley's aster	Stratification
Aster ericoides L.	Tufted white prairie aster	Stratification
Geum triflorum Pursh	Three-flowered avens	Stratification
Helianthus subrhomboideus Rydb.	Rhombic-leaved sunflower	Stratification
Heterotheca villosa (Pursh) Shinners	Hairy golden aster	Stratification
Liatris punctata Hook.	Dotted blazingstar	Stratification
Solidago rigida L.	Stiff goldenrod	Stratification
Native Grasses		
Agropyron dasystachyum (Hook.) Scribn.	Northern wheatgrass	Stratification
Agropyron smithii Rydb	Western wheatgrass	Stratification
Agropyron trachycaulum var. *trachycaulum* (Link) Malte	Slender wheatgrass	Stratification
Bouteloua gracilis (HBK.) Lag.	Blue grama	Stratification
Bromus ciliatus L.	Fringed brome	Stratification
Festuca hallii (Vasey) Piper	Plains rough fescue	Stratification
Koeleria macrantha (Ledeb.) L.A. Schultes	June grass	Stratification
Stipa comata Trin. & Rupr.	Needle-and-thread	Acid scarification[2] & stratification
Stipa curtiseta (A.S. Hitchc.) Barkworth	Porcupine grass	Stratification
Stipa viridula Trin.	Green needle grass	Acid scarification & stratification
Native Legumes		
Astragalus crassicarpus Nutt.	Ground plum	Mechanical scarification[3]
Astragalus striatus Nutt.	Ascending purple milk-vetch	Mechanical scarification
Glycyrrhiza lepidota (Nutt.) Pursh	Wild licorice	Mechanical scarification

Table 4.2, continued

Scientific Name	Common Name	Seed Treatment
Hedysarum alpinum L.	Hedysarum	Mechanical scarification
Oxytropis monticola A. Gray	Late yellow locoweed	Mechanical scarification
Psoralea esculenta Pursh	Indian breadroot	Mechanical scarification
Thermopsis rhombifolia (Nutt.) Richards.	Goldenbean	Mechanical scarification & stratification

[1] Moistened filter paper 5 °C, min. 14d
[2] 1N HCl 8-10 min.
[3] Abrasion with sandpaper ~1 min.

at least 14 days. If no literature on optimal seed treatments for a species was found, seeds were stratified for two weeks as described above, as many northern species benefit from this treatment (Blake, 1935; Smreciu et al., 1988). Seed germination was assessed using a standard test on wet filter paper (22° C, 28 d) (Nernberg, 1994) before use in growth chamber experiments. Western wheatgrass, needle-and-thread, porcupine grass and green needlegrass, were not tested, as they will not germinate on filter paper (Nernberg, 1994).

Plastic trays (25.4 x 52 x 5.7 cm) were filled with 2 kg of soil 1 or 2.5 kg of soils 2 and 3 for each treatment (control, 1000, 5000, 10000 and 50000), or 2.4 kg of field soils. Different amounts of soil were added because of differences in bulk density. Ten seeds (n=10) of six species were planted in each of six rows, about 5 cm apart, in each tray. The flats were placed in a Conviron (Winnipeg, MB) growth chamber receiving 16 hours light at 25 °C (±0.5 °C) and 8 hours dark at 15 °C (±0.5 °C). Lighting was provided by moveable banks of fluorescent and incandescent lights providing approximately 50,000 lux. These conditions were chosen to approximate optimal temperature and light conditions in Saskatchewan in summer. The soil was kept damp

by misting with distilled water when the surface of the soil was dry. Emergence from the soil and survival was recorded weekly for five weeks. A plant was considered to have survived if the leaves were green and turgid.

4.3.2 Productivity and relative growth rate screening

The twelve species selected for the productivity and RGR screening had 60% or greater survival in at least three of the soils contaminated with 10,000 or 50,000 ppm crude oil (wt/wt) or the contaminated field soil. Only one soil was used in this screening due to limited growth chamber space so that more replicates could be grown. Soil 2 was selected because plant survival was higher than in soil 3 but organic matter content lower than in soil 1. Lower organic matter was desired as it may adsorb crude oil thus affecting bioavailability (Cunningham et al., 1996). Soil 2 was contaminated with crude oil in amounts equaling 5,000, 10,000 and 50,000 ppm (wt/wt) using the same procedure as for survival screening (section 4.3.1).

An experimental unit consisted of a single seed sown in a 4 x 20 cm cone-tainer containing 116 g of soil (Stuewe and Sons, Corvallis, OR). The experimental design was completely randomized with 21 replicates. The cone-tainers were placed in a Conviron (Winnipeg, MB) growth chamber receiving 16 hours light at 25 °C (±0.5 °C) and 8 hours dark at 15 °C (±0.5 °C). Lighting was provided by moveable banks of fluorescent and incandescent lights providing approximately 50,000 lux. The container capacity (i.e. the amount of water held by soil in a container, initially saturated with water and then allowed to drain) was determined (Cassel and Nielsen, 1986). Blake (1935) indicated that one-half to two-third saturation was optimal for germination of native prairie plants so soils were kept at 60% of container capacity by watering daily to

a predetermined weight. However, in the 50000 treatment water sometimes dripped out the drainage holes, likely due to a lower water-holding capacity. The emergence date was recorded and each plant grown for 28 days after emergence. Aerial and root biomass were harvested after four weeks. Roots were removed by gently shaking the soil off and picking up any broken pieces with forceps. The roots were then washed with distilled water to remove clinging soil particles, dried at 60 °C for 24-26 hours, and weighed. RGR was calculated using the following formula (Fitter and Hay, 1987):

$$RGR = \frac{\ln W_2 - \ln W_1}{t} \qquad [4.1]$$

where W_2 = mean oven dry seedling mass at harvest, W_1 = mean oven dry initial seedling mass and t = length of the growing time (4 weeks). Initial seedling mass was determined by harvesting seedlings (n=35) 12 hours after germination on moist filter paper in Petri dishes and oven drying at 60 °C for 24 hours. Relative performance (RP) was calculated using the following formula (Kulakow et al., 2000):

$$RP = \frac{Wc}{Wu} * 100 \qquad [4.2]$$

where W_C is the mean oven-dry mass of the plants grown in contaminated soil at harvest and W_U is the mean oven-dry mass of the plants grown in uncontaminated soil.

4.3.3 Statistical analyses

For the survival screening experiment, species were ranked according to percentage survival. A Kruskal-Wallis test with significance of $P \leq 0.05$ was performed to detect rank differences between the soil treatments. Analysis of variance (ANOVA) with significance of $P \leq 0.05$ and Tukey's LSD was performed for emergence time, total

and root biomass and RGR. Correlation analyses between RGR in uncontaminated soil and total biomass in contaminated soils were conducted. Logarithmic transformations to homogenize variances were applied to seedling biomass. All statistical tests were done using MINITAB software (MINITAB Inc., State College, PA).

4.4 Results

4.4.1 Survival screening

Pure live seed (PLS) data on the entire seed lot of each species was obtained from the supplier (Table 4.3). In general the PLS was higher for non-native plant species than native ones. Eighteen species, including all of the non-natives and 11 natives had 60% or greater survival in soil 1 contaminated with 10,000 ppm crude oil; this decreased to ten species in the 50,000 ppm crude oil treatment. Survival was lower in soils 2 and 3 with only twelve and five species, respectively, having 60% or higher survival in the 1% treatment. In the 50,000 ppm treatment, only four species had 60% or higher survival in soil 2, and none in soil 3. In the contaminated field soil, nine species had 60% or greater survival. Low survival in all soils was due to low emergence not high mortality. Seedling mortality was less than 6% in all soil treatments except the 50,000 ppm treatment of soil 3, where it reached 50%. Survival of many native species was low in control as well as contaminated soils, suggesting that factors other than crude oil were responsible. The four soils were not significantly different (P=0.935) mean emergence rank. Thus survival in all four soils was similar with ranked species, even though the absolute survival values were different.

Table 4.3. Percentage pure live seed, germination in standard petri dish test (n=75), survival 35 days after seeding in four uncontaminated and crude oil-contaminated soils (n=10) and mean survival rank in all treatments.

			Survival in Crude Oil Treatments (%) [a]											Mean
	PLS[b]	Germination	Soil 1			Soil 2			Soil 3			Field Soil		Survival
Plant Species	%	%	0%	1%	5%	0%	1%	5%	0%	1%	5%	0%	2%	Rank[c]
Non-native Grasses														
Agropyron pectiniforme [+d]	95	92	80	70	0	40	60	10	70	10	10	80	90	15
Bromus inermis [+]	96	75	80	80	20	80	60	50	30	40	0	60	80	12.5
Phleum pratense [+]	94	75	70	80	30	40	60	30	40	40	0	70	70	14
Poa pratensis [+]	91	31	50	70	50	80	60	10	70	50	50	80	60	12.5
Non-native Legumes														
Medicago sativa [+]	95	85	90	70	70	80	80	60	50	40	20	80	80	3
Melilotus officinalis [+]	95	77	80	100	100	70	60	50	70	40	0	80	40	5
Trifolium repens [+]	98	95	90	60	80	80	100	90	70	90	20	80	90	1
Native Forbs														
Achillea millefolium	80	0	0	0	0	0	0	0	70	60	0	0	0	35
Artemisia frigida [+]	95	96	70	100	100	90	60	40	70	70	10	30	40	4
Aster ciliolatus	70	0	10	0	0	0	0	0	0	10	0	0	10	38
Aster ericoides	81	19	20	0	30	80	70	10	40	0	0	20	20	24
Gaillardia aristata	82	21	10	20	20	10	10	10	30	10	0	30	0	29
Galium boreale	80	19	20	20	0	40	10	0	10	20	0	10	0	34
Geum triflorum	85	80	60	80	50	80	20	20	80	50	30	10	10	10.5

Table 4.3, continued

Plant Species	PLS[b] %	Germination %	Survival in Crude Oil Treatments (1%) [a] Soil 1 0%	Soil 1 1%	Soil 1 5%	Soil 2 0%	Soil 2 1%	Soil 2 5%	Soil 3 0%	Soil 3 1%	Soil 3 5%	Field Soil 0%	Field Soil 2%	Mean Germination Rank[c]
Helianthus subrhomboides	80	21	10	20	10	20	20	10	10	10	10	10	0	28
Heterotheca villosa	80	29	30	20	30	40	10	10	70	50	0	0	0	17.5
Liatris punctata	81	20	10	0	10	10	20	40	0	0	0	30	0	32
Linum lewisii	85	73	50	100	20	60	40	20	0	40	0	70	20	17.5
Potentilla pensylvanica [+]	90	85	80	100	100	80	70	30	70	60	10	90	100	2
Ratibida columnifera	80	45	40	20	80	0	40	10	30	0	0	0	30	27
Solidago rigida	82	71	60	30	30	30	40	80	80	0	10	80	20	10.5
Native Grasses														
Agropyron dasystachyum	85	13	0	10	0	0	0	10	30	0	0	0	20	39
Agropyron smithii	80	N/A	30	0	0	10	10	0	10	0	0	0	0	37
Agropyron trachycaulum	85	44	30	50	10	40	30	10	40	0	0	60	10	22
Bouteloua gracilis	75	32	20	30	20	40	50	20	10	0	0	0	20	21
Bromus ciliatus [+]	75	36	90	80	70	80	70	10	90	20	0	80	20	8
Calamagrostis canadensis	N/A[e]	77	60	40	10	70	20	0	20	20	0	40	20	23
Festuca hallii	85	3	10	30	0	0	50	10	10	40	0	10	20	30
Koeleria macrantha	90	49	50	40	30	30	30	0	50	30	0	30	20	20
Stipa comata	82	N/A	40	70	30	10	0	0	0	0	0	30	40	31
Stipa curtiseta	80	N/A	30	20	10	30	30	0	0	0	0	0	10	36

Table 4.3, continued

			Survival in Crude Oil Treatments (%) [a]											Mean
	PLS[b]	Germination	Soil 1			Soil 2			Soil 3			Field Soil		Germination
Plant Species	%	%	0%	1%	5%	0%	1%	5%	0%	1%	5%	0%	2%	Rank[c]
Stipa viridula	85	N/A	70	60	10	0	0	0	0	0	0	30	50	33
Native Legumes														
Astragalus crassicarpus	80	20	30	20	0	30	50	40	10	0	0	20	0	25
Astragalus striatus	78	27	60	100	60	50	50	20	40	50	10	70	50	7
Glycyrrhiza lepidota [+]	86	56	90	100	100	20	40	60	70	30	10	0	0	9
Hedysarum alpinum	85	57	40	60	10	30	20	20	10	50	10	60	40	16
Oxytropis monticola	70	7	0	10	0	10	10	0	20	60	50	60	100	26
Psoralea esculenta [+]	95	99	100	90	90	60	60	50	40	40	20	60	90	6
Thermopsis rhombifolia	85	29	0	30	50	80	40	10	30	0	10	30	50	19

[a] Percentage crude oil (wt/wt)
[b] Pure live seed of the seed lot
[c] Mean survival rank for three treatments (i.e. control, 1%, 2% and 5%) of all four soils
[+d] Species selected for biomass screening experiment
[e] Data not available

4.4.2 Productivity and relative growth rate screening

The mean number of days until emergence increased as the concentration of crude oil increased (Figure 4.1). However, the difference was significant in only five of the eleven species: crested wheatgrass, alfalfa, prairie cinquefoil, Indian breadroot and white clover. Emergence of pasture sage occurred in less than five days in all treatments. Emergence of Kentucky bluegrass took the longest, more than ten days on average in all treatments. In the 50,000 ppm treatment the mean number of days before emergence was at least ten for eight of the eleven species. Due to low emergence (less than 5% in all treatments) data for the twelfth species, wild licorice, were not presented. When the seeds of wild licorice that had not emerged were recovered, the seeds showed evidence of a fungal infection.

Fringed brome produced the highest total biomass in control soil, but not in the contaminated soils (Figure 4.2): its biomass was reduced by more than half in all three crude oil treatments. The addition of any amount of crude oil significantly decreased total biomass produced by all species except Indian breadroot, which produced the most biomass of all species in the contaminated treatments. The native forbs, prairie cinquefoil and pasture sage, produced the lowest total biomass in all treatments. In general, three plant response patterns were evident: (i) biomass production was unaffected by crude oil (e.g. Indian breadroot); (ii) biomass production dropped steadily as the concentration of crude oil increased (e.g. crested wheatgrass); or (iii) biomass production was similar in two crude oil treatments, but dissimilar from the third (e.g. smooth brome, yellow sweet-clover). The relative performance of all species except

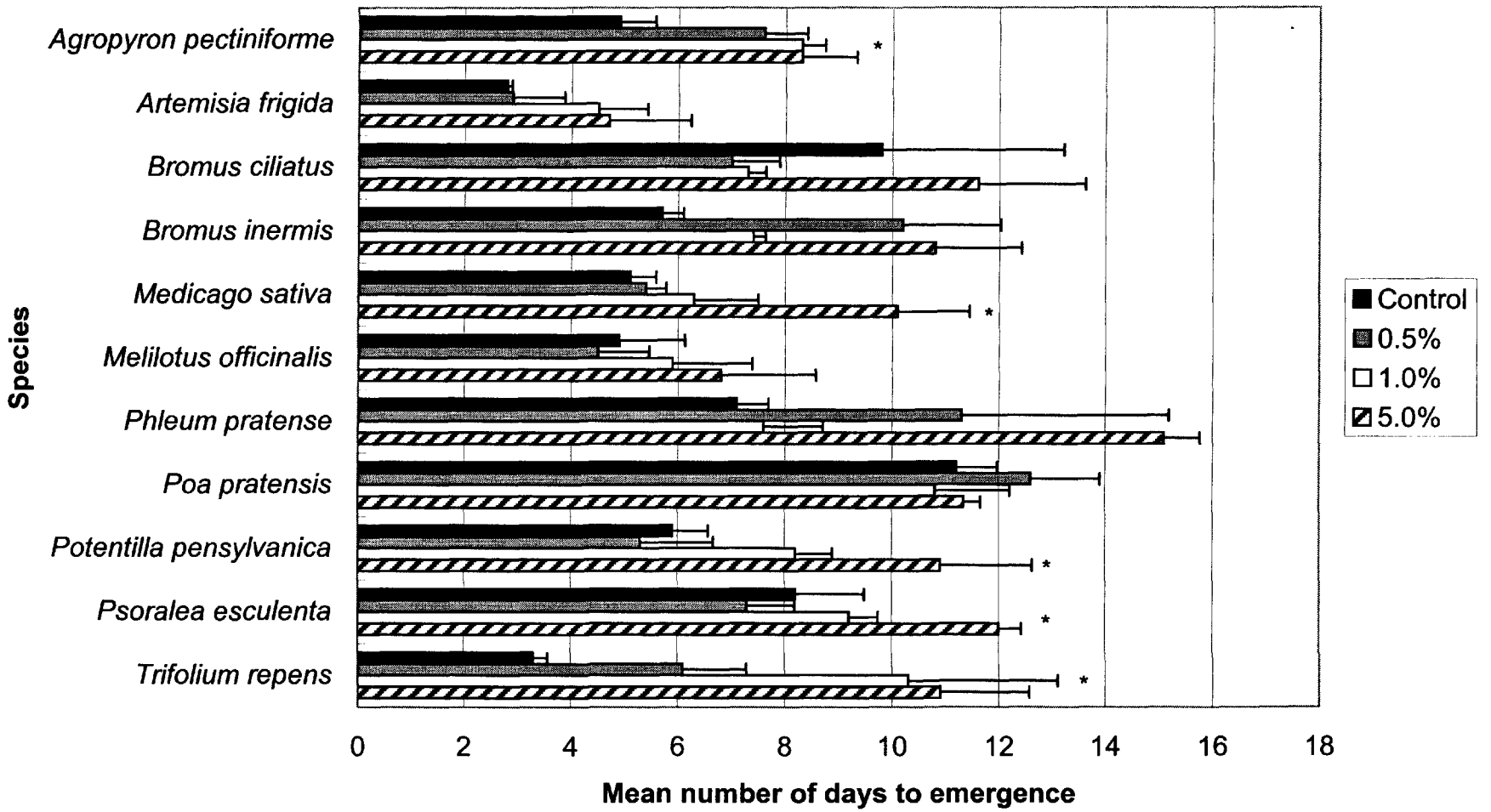

Figure 4.1. Mean number of days to emergence of 11 plants native or non-native in western Canada in uncontaminated and crude-oil contaminated soil. Statistically significant ($P \leq 0.05$) differences in values within species using ANOVA and Tukey's LSD denoted by *. Error bars represent SE (minimum n=7, maximum n=20).

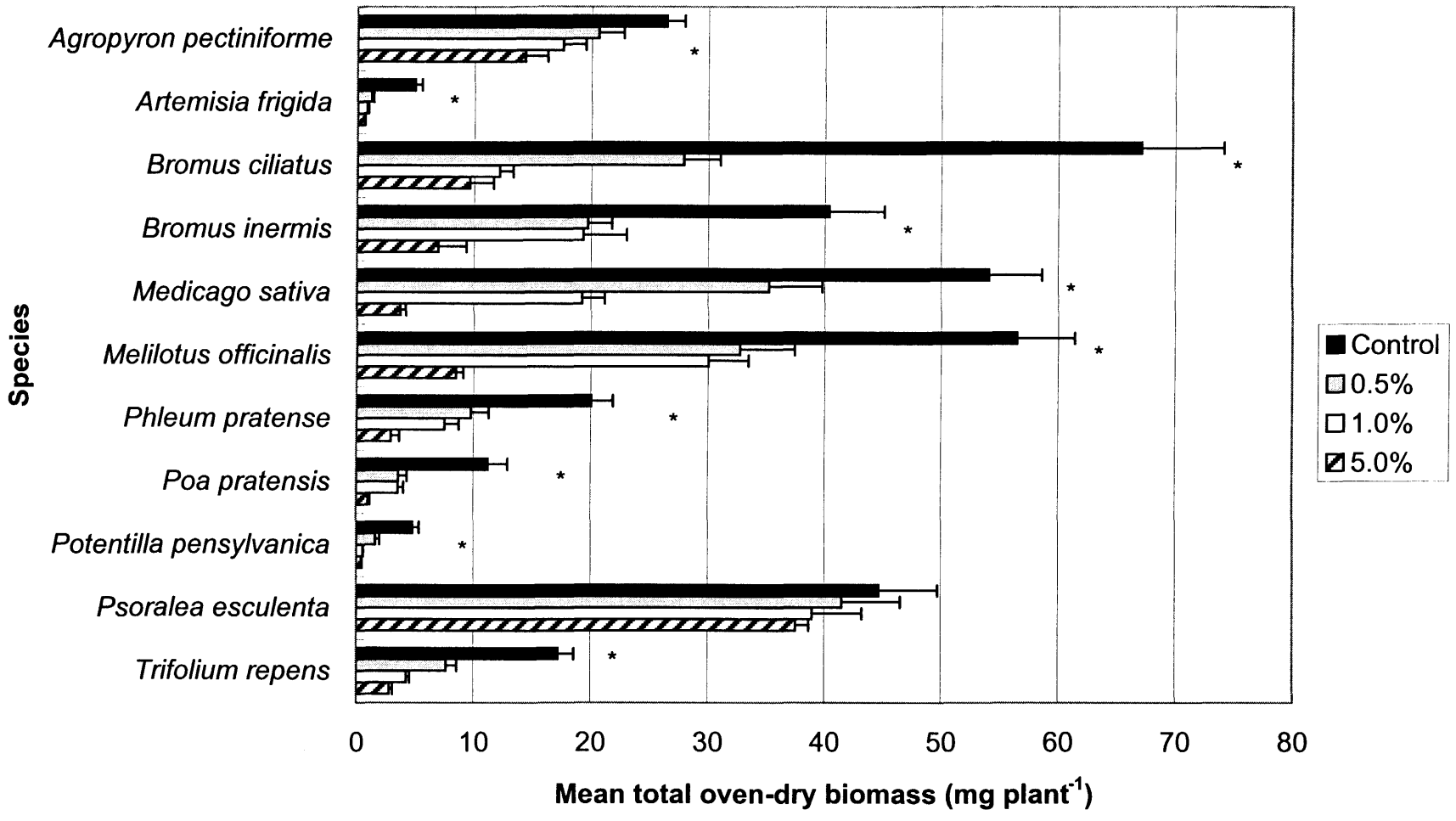

Figure 4.2. Mean total oven-dry biomass of 11 plants native or non-native in western Canada 28 days after emergence in uncontaminated and crude-oil contaminated soil. Statistically significant ($P \leq 0.05$) differences in values within species using ANOVA and Tukey's LSD denoted by *. Error bars represent SE (minimum n=7, maximum n=20).

Indian breadroot and crested wheatgrass was less than 20% in the 50,000 ppm crude oil treatment (Figure 4.3).

Reductions in root biomass with crude oil addition had a similar pattern as total biomass with one exception; root biomass was not significantly different in pasture sage (Figure 4.4). This suggests that pasture sage is capable of switching its carbon allocation to roots under stressful conditions. Indian breadroot produced the most root biomass, about 16 mg, in the 50,000 ppm crude oil treatment. Smooth brome, yellow sweet-clover and crested wheatgrass produced similar root biomass in the 5,000 and 10,000 ppm treatments but only crested wheatgrass produced root biomass over 10 mg in the 5% treatment. Relative performance was less than 25% in all species except Indian breadroot, crested wheatgrass and pasture sage (Figure 4.5).

Species with the lowest RGR in the control soil were Indian breadroot and crested wheatgrass (Figure 4.6). The species with highest RGR in control soil, pasture sage and prairie cinquefoil, were the ones with the lowest total and root biomasses (Figures 4.2 and 4.3). The remainder of the species had relatively high RGR, between 0.6 and 1.0. As crude oil concentrations increased, RGR significantly declined in all species except Indian breadroot. The relationship between RGR in control soil and the corresponding seedling biomass in three crude oil treatments is shown in Figure 4.7. There is a significant ($P \leq 0.05$) correlation between seedling biomass and RGR in all crude oil treatments. The correlation becomes stronger as crude oil concentration increases from 5,000 to 50,000 ppm. The correlation coefficient between RGR and the seedling biomass in control soil was not significant (r= -0.4278) (data not shown).

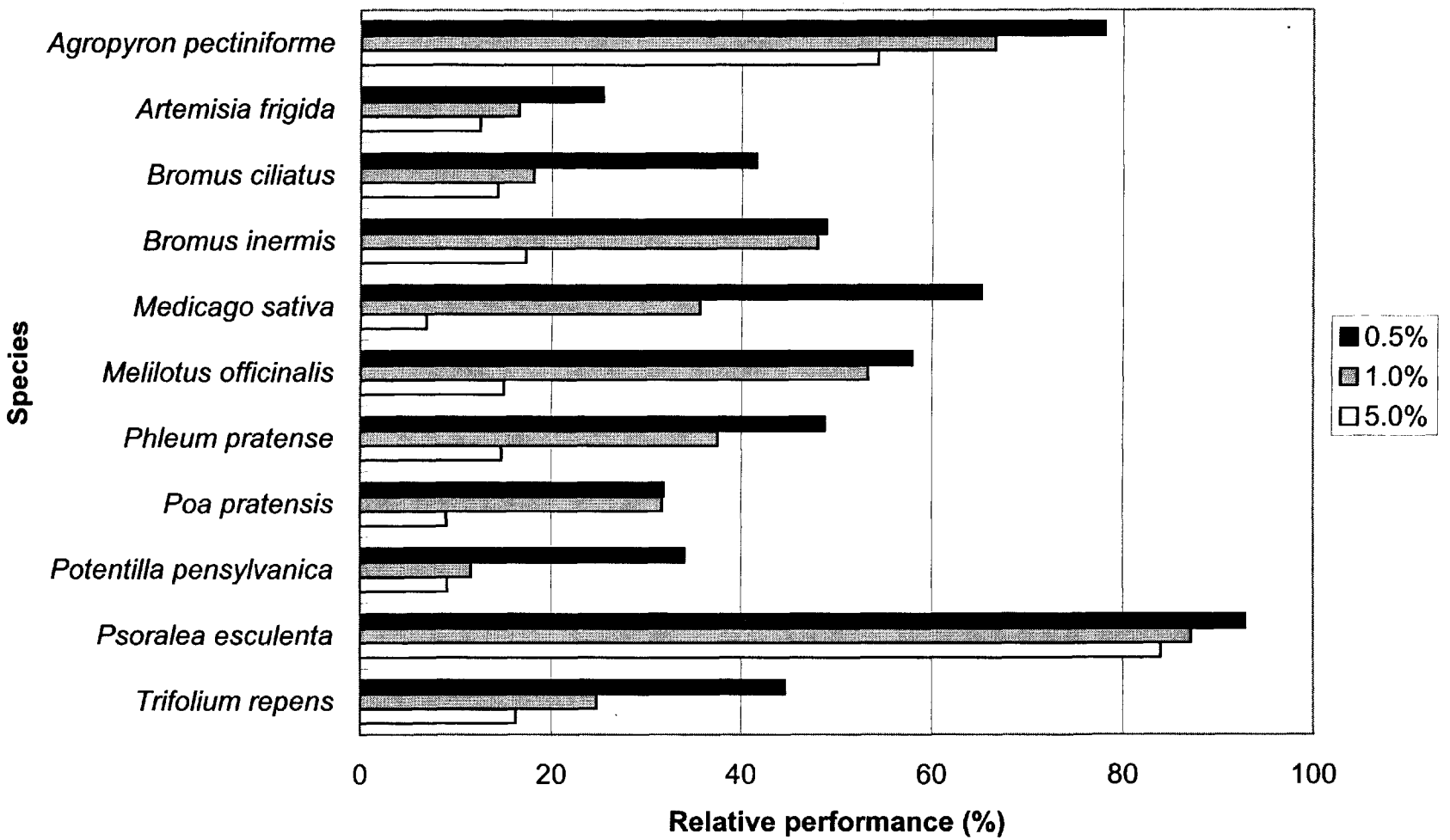

Figure 4.3. Relative performance for total biomass of 11 plants native or non-native in western Canada 28 days after emergence in crude-oil contaminated soil.

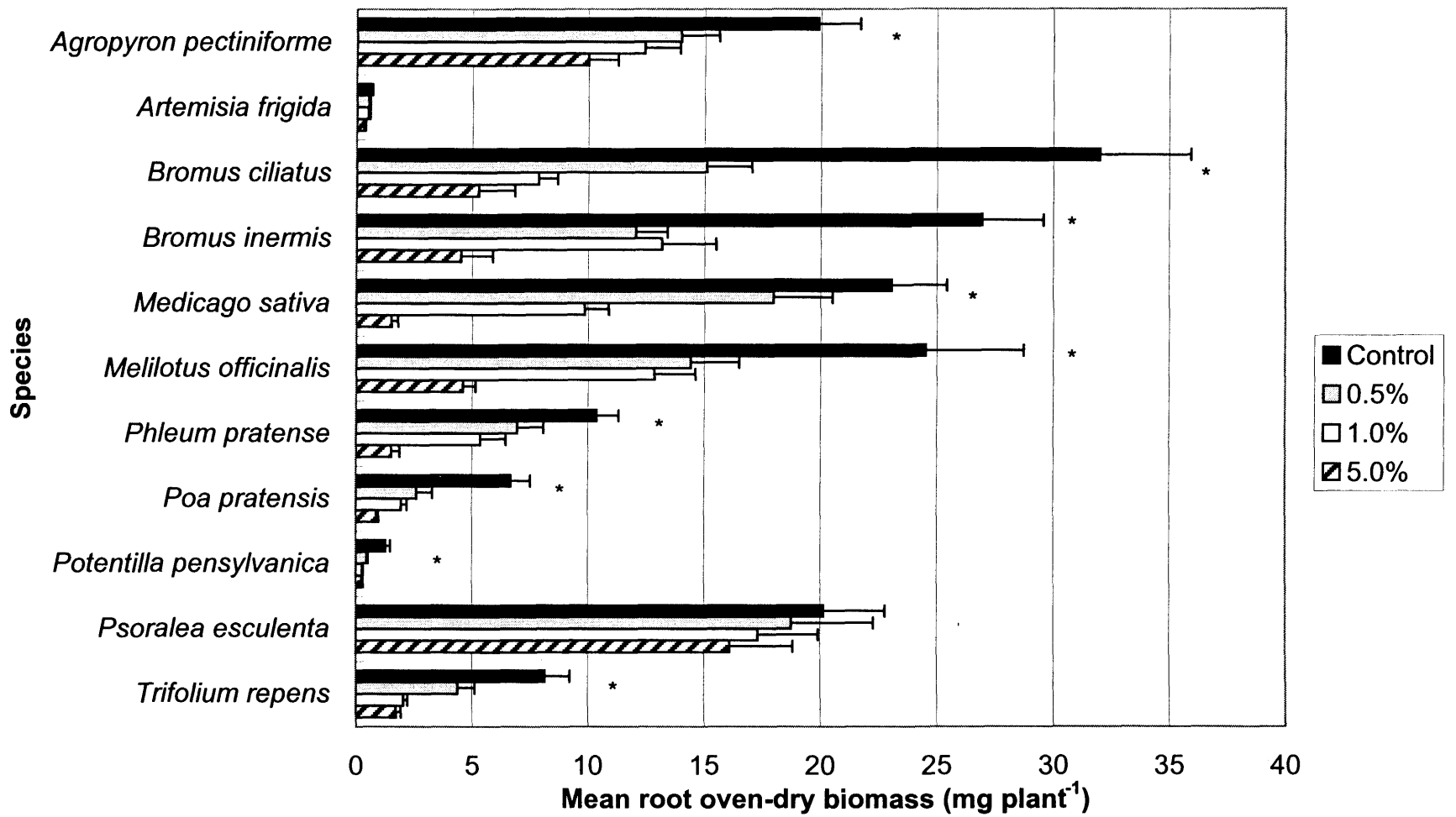

Figure 4.4. Mean root oven-dry biomass of 11 plants native or non-native in western Canada 28 days after emergence in uncontaminated and crude-oil contaminated soil. Statistically significant ($P \leq 0.05$) differences in values within species using ANOVA and Tukey's LSD denoted by *. Error bars represent SE (minimum n=7, maximum n=20).

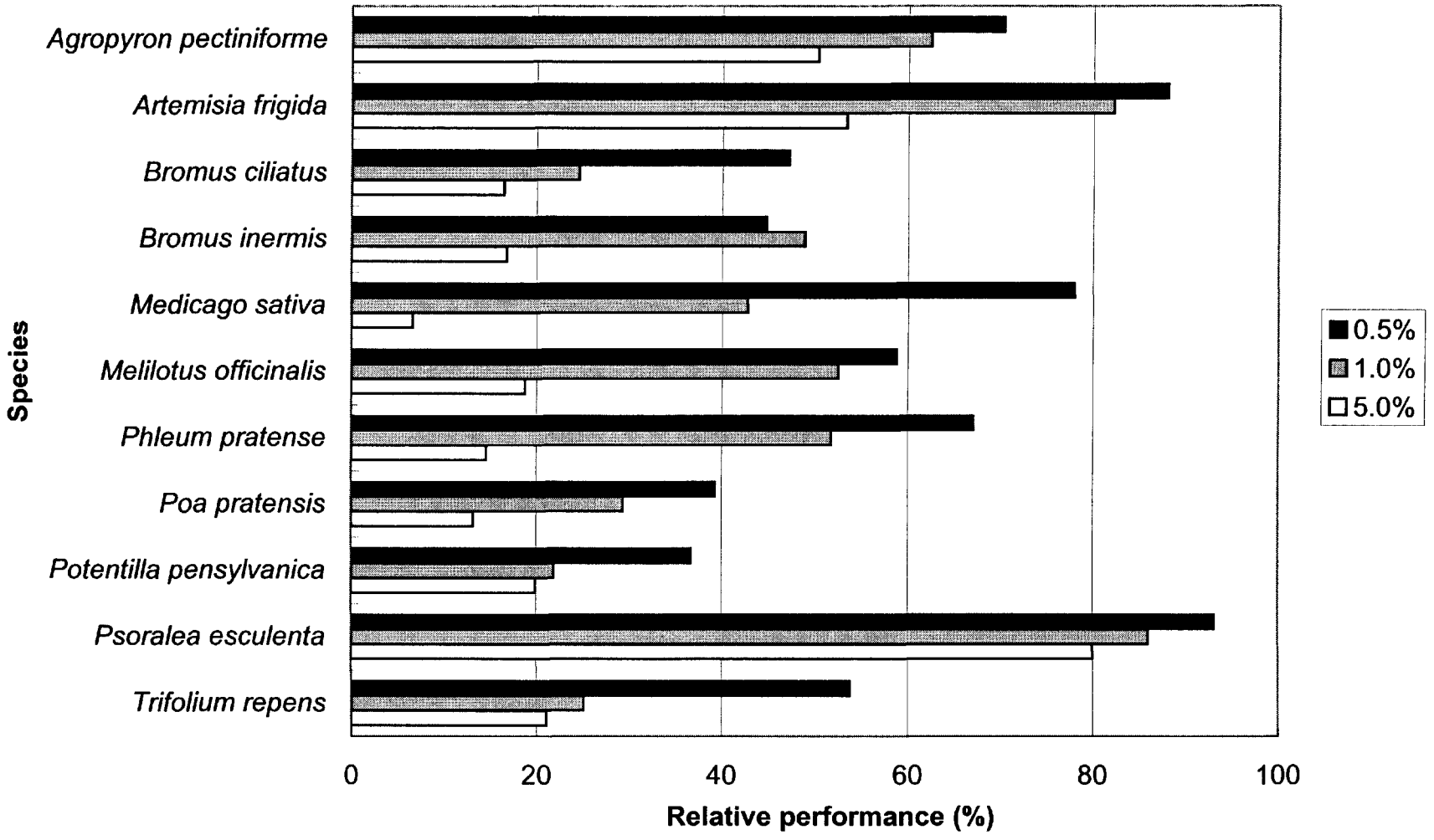

Figure 4.5. Relative performance for root biomass of 11 plants native or non-native in western Canada 28 days after emergence in contaminated soil.

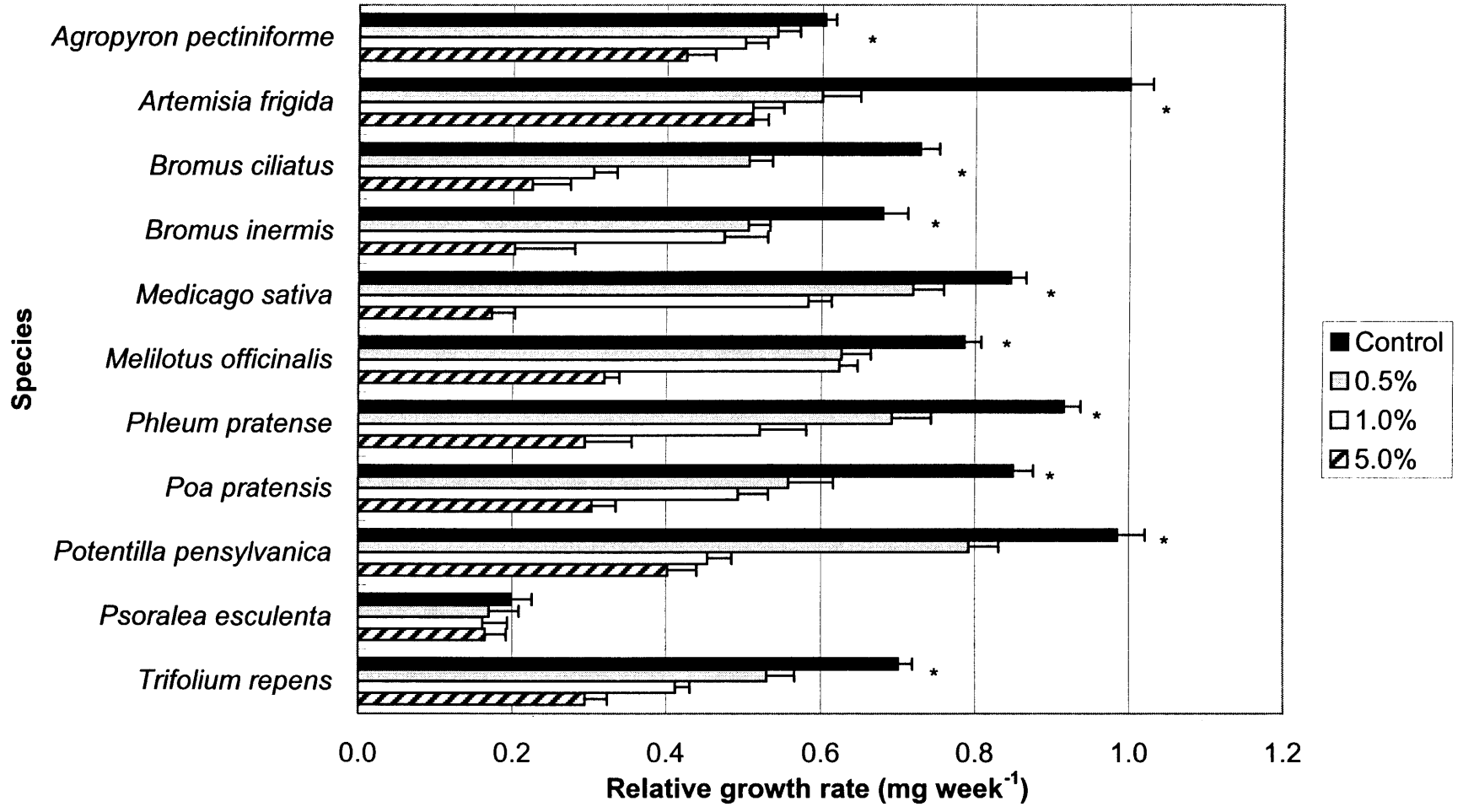

Figure 4.6. Mean relative growth rate of 11 plants native or non-native in western Canada 28 days after emergence in uncontaminated and crude-oil contaminated soil. Statistically significant ($P \leq 0.05$) differences in values within species using ANOVA and Tukey's LSD denoted by *. Error bars represent SE (minimum n=7, maximum n=20).

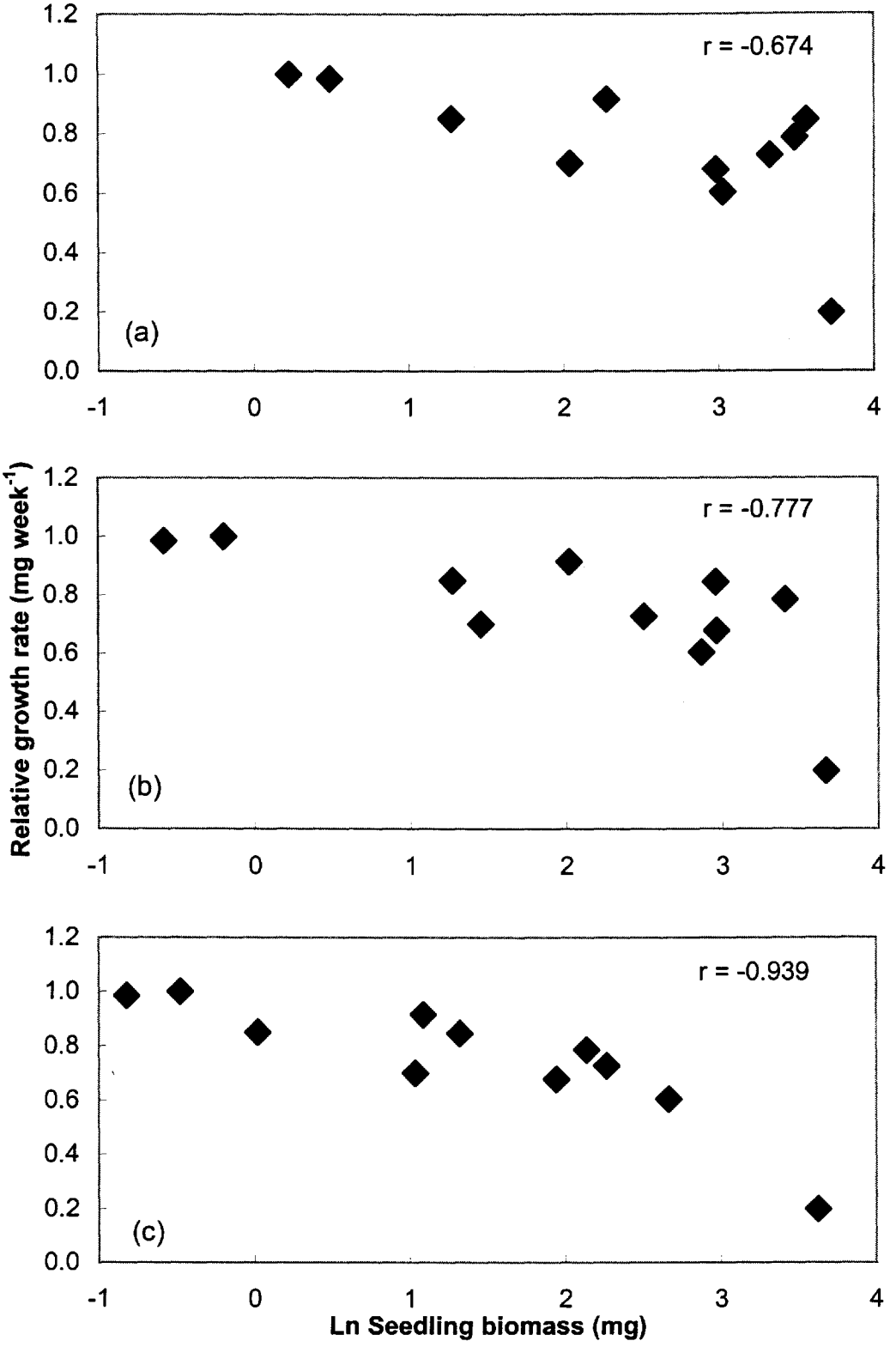

Figure 4.7. Relationship between relative growth rate in control soil and seedling biomass in (a) 0.5% (b) 1% and (c) 5% crude oil treatments. All correlations were significant at $P \leq 0.05$.

4.5 Discussion

This chapter describes several experiments that tested the ability of native and non-native plants to tolerate crude oil-contaminated soil. The relationship between biomass production and RGR was examined, as it has been suggested that plants with low RGR may be more stress-resistant (Elias and Chadwick, 1979). The twelve species with the highest survival varied greatly in their tolerance; some species exhibited dramatic declines in biomass and RGR with increasing concentrations of crude oil while others were hardly affected. The reason for this variation appears to be related to RGR. Those species with the lowest RGR in uncontaminated soil were the ones exhibiting the least change in biomass with crude oil addition and visa versa. Thus this research supports the hypothesis of Elias and Chadwick (1979) that RGR can be used as a way of screening species for reclamation purposes, as it is indicative of stress-tolerance.

Grime's (1979) theory regarding plant strategies explains the responses to crude oil observed in this and other studies. Plants employ three basic resource use strategies: stress-tolerant (S), competitive (C) or ruderal (R) (Grime, 1979). Stress-tolerant species have low RGR, which they can maintain despite exposure to stressful conditions, such as infertile soil (Shipley and Keddy, 1988; van der Werf et al., 1993). In contrast, plants using C or R strategies typically have high RGR to help them capture resources in fertile habitats (Poorter and Garnier, 1999). In nutrient-poor habitats C and R species exhaust soil nutrients (Poorter and Garnier, 1999). Our results suggest that Indian breadroot is stress-tolerant, as it has low RGR and was able to maintain its normal growth when faced with a stressful habitat (crude oil-contaminated soil). Based on its tolerance to crude oil, and second lowest RGR (about 0.6), crested wheatgrass likely

uses a C-S strategy. Pasture sage, which has been classified as a C-R (Bai, 1993), had a high RGR and severely reduced biomass production when faced with stress (i.e. hydrocarbons in soil). The high RGR of prairie cinquefoil suggests that it is also a C-R. Grime and associates (1990) classify timothy, Kentucky bluegrass and white clover as C-S-R. The rest of the species also likely use a C-S-R strategy given that their RGRs are between 1 and 0.6. Grime's theory (1979) may also explain the results of Kulakow and associates (2000). After 180 days growing in contaminated soil, red fescue (*Festuca rubra* L.) had the highest root biomass and barley (*Hordeum vulgare* L.) the lowest. Red fescue is a slow-growing species that is classified as C-S-R with some populations tending to C, C-S or S due to genetic variability (Grime et al., 1990). Barley on the other hand is a C-R species with a high RGR (Grime, 1979). Plants that are most tolerant of crude oil-contaminated soil will likely use an S, C-S, S-R or C-S-R strategy. Plants using C and R strategies will probably fare poorly in crude oil-contaminated soil as nutrient conditions are not favourable for their rapid growth.

One of the major questions raised by this study is what caused the highly variable survival of many native species, which resulted primarily from low emergence, not high mortality. An examination of the literature indicates that there are many factors other than the presence of crude oil that could have affected emergence. Native plants often have low germination because of dormancy mechanisms to ensure germination in a suitable habitat at an opportune time (Blake, 1935). Although seed treatments were applied to break dormancy, optimal seed treatments for some species were unknown (e.g. northern bedstraw (*Galium boreale* L.), rhombic-leaved sunflower (*Helianthus subrhomboideus* L.). Not applying proper seed treatments to native plants

can dramatically reduce germination (Blake, 1935). Unfortunately, the only seed treatment recommendations that could be found for some species were in technical manuals as opposed to science-based research results. Thus, whether some of the seed treatments applied were truly optimal is unknown.

Even with recommended seed treatment, some native species exhibit low or delayed germination (Greene and Curtis, 1950; Smreciu et al., 1988). Environmental conditions at the time of seed development, seed maturity, age of seed and seed storage conditions can affect germination. Seed development may be impaired if soil fertility or moisture is low when seeds are developing (Pahl and Smreciu, 1999). If seeds are harvested before they are fully mature, germination is typically lower (Young and Young, 1986). Seed viability typically declines with age of the seed, in some species more rapidly than in others (Blake, 1935). Seeds stored under refrigerated conditions will retain their viability longer than if they were stored at room temperature (Young and Young, 1986). These factors resulted in a lower percentage PLS in native compared to non-native species, affecting the emergence. Furthermore, some species were more susceptible to fungal infection than others (i.e., goldenbean (*Thermopsis rhombifolia* (Nutt.) Richards.), wild licorice), which prevented emergence.

Another important point to consider is that native grassland plants attain optimal germination over a wide range of temperatures, moisture conditions, soil textures and fertility. In general, species germinate best under temperature and moisture conditions most favorable for their adult growth (Young and Young, 1986). For example, C4 grasses had higher germination than C3 grasses under warm temperatures with high water stress (Qui, 1988). Germination of the native grass Canby bluegrass was

significantly lower in clay soil than sandy loam soil (Evans et al., 1977). This may be due to the effect that texture has on water holding capacity (Bannister, 1964; de Alba-Avila and Cox, 1988). As organic matter also affects water-holding capacity of soils (Baldock and Nelson, 2000), it is likely that this variable affects germination. Nitrate is required by some species to promote germination: adding nitrate improves germination in the native grasses dropseed (*Sporobolus asper* (Beauv.) Kunth) and alkali sacaton (*S. airoides* (Torr.) Torr.) (Toole, 1941). It is possible that the conditions plants were grown under in this study were suboptimal for germination of certain species, as optimal conditions were usually unknown.

Where survival was low in uncontaminated and contaminated soils (e.g. dotted blazingstar (*Liatris punctata* Hook.), western and northern wheatgrass etc.), conclusions about the species' ability to tolerate crude oil cannot be made. Experiments using different seed stock, seed treatments or environmental conditions may reveal that these species are in fact tolerant of hydrocarbons. To improve germination of native plants, scientifically proven seed treatments should be applied. If no studies addressing optimal seed treatments have been conducted, experiments to determine this should be initiated. Germination, particularly that of legumes, may be improved with seed sterilization or application of a fungicide (Young and Young, 1986). Temperature and watering regimes should be varied and different soil textures used if there is a large difference in optimal conditions for germination among species being tested. Both non-native and native plants must be given more time to germinate when testing for hydrocarbon tolerance as one effect of hydrocarbons on plants is delayed germination (Baker, 1970; Amakiri and Onofeghara, 1984). In this study the mean germination of

eight species was greater than 10 days in the 5% treatment; some individuals did not germinate until 20 days after planting. Furthermore, large numbers of seeds need to be tested due to the low PLS of many native species. Determining the PLS of seed lots can help in assessing if seed treatment or low viability is affecting germination.

4.6 Conclusions

The presence of fresh crude oil in soil inhibited emergence of many species, more so in the soil with the lowest organic matter. The ten species with the highest emergence ranks in all treatments were, in descending order: white clover, prairie cinquefoil, alfalfa, pasture sage, yellow sweet-clover, Indian breadroot, ascending purple milk-vetch, fringed brome, wild licorice and three-flowered avens. Due to the low emergence of some native species in uncontaminated soil, conclusions about their ability to tolerate crude oil cannot be made. The presence of crude oil significantly delayed emergence, and reduced the biomass and relative growth rate of most of the 12 species tested. The RGR in uncontaminated soil was strongly correlated with biomass production in soil contaminated with 50,000 ppm crude oil. The species with the lowest RGR in uncontaminated soil, Indian breadroot and crested wheatgrass, were the least affected by the crude oil in terms of their biomass production. More research is needed to determine if this correlation occurs in a wider number of species.

5.0 EFFECT OF SEED SIZE AND RELATIVE GROWTH RATE ON HYDROCARBON TOLERANCE IN SELECTED PLANT SPECIES

5.1 Abstract

The seedling mass and relative growth rate responses of eight plant species to uncontaminated and crude oil-contaminated soil were compared. These results permit testing the hypothesis that seed mass of a species determines its sensitivity to hydrocarbons. The species with larger seeds had smaller reductions in seedling mass and relative growth rate in contaminated compared to uncontaminated soil than those with smaller seeds. Species with the highest relative growth rates in uncontaminated soil had the lowest seedling mass in contaminated soil. Seed mass and relative growth rate were negatively correlated. These results suggest that species with larger seeds may be more tolerant of crude oil-contaminated soil than small seeded species.

5.2 Introduction

Phytoremediation is the use of plants and their associated microorganisms to contain, sequester or degrade inorganic contaminants like heavy metals and organic contaminants like petroleum hydrocarbons in soil (Cunningham and Ow, 1996). Studies note that the presence of plants enhances hydrocarbon degradation in soil (Aprill and Sims, 1990; Schwab et al., 1995; Reilley et al., 1996). The first step in phytoremediation is to identify plants adapted to climatic conditions at the contaminated

site that can also tolerate hydrocarbons. To date, screening plants for hydrocarbon tolerance has been done by growing plants in contaminated soil in greenhouses or growth chambers (Kulakow et al., 2000), or observing which species colonize contaminated sites naturally (Olson and Fletcher, 2000). Some species are more tolerant of hydrocarbons in soil than others (Xu and Johnson, 1995; Chaîneau et al., 1997; Kulakow et al., 2000), but the reason why has not been determined. An explanation for hydrocarbon tolerance in plants would aid in phytoremediation screening.

The relationship between relative growth rate (RGR), seed mass and seedling mass has been the subject of study by ecologists since the 1970s. Large-seeded species with low RGR are generally more tolerant of arid (Jurado and Westoby, 1992; Leishman and Westoby, 1994a), shady (Leishman and Westoby, 1994b) and low nutrient (Hanley and Fenner, 1997; Milberg et al., 1998) conditions although there are some exceptions (Fenner and Kitajima, 1999; Poorter and Garnier, 1999). Weinstein and Yanai (1994) note that plants with low RGRs are more tolerant of ground level ozone than those with high RGRs. Heavy metal tolerant plants also tend to have low RGRs, although this may be due to the infertility of habitats high in heavy metals (Lambers and Poorter, 1992). The effect that seed mass and RGR might have on the ability of plants to grow in hydrocarbon-contaminated soil has not been explored. The addition of petroleum hydrocarbons to soil increases the C:N:P ratio resulting in immobilization of N and P by microorganisms (Udo and Fayemi, 1975; Xu and Johnson, 1997). Thus hydrocarbon contamination decreases soil fertility by reducing plant available N and P. Furthermore hydrocarbons in soil inhibit nutrient uptake by

coating plant roots (Baker, 1970; Xu and Johnson, 1997). Since large seed mass and low RGRs may be advantageous in soils of low fertility, large seeded species may be more tolerant of hydrocarbon-contaminated soil that is low in available nutrients (Udo and Fayemi, 1975) than small seeded species.

This study was conducted to test the hypotheses that: (1) large seeded plant species are more tolerant of hydrocarbon-contaminated soil than small seeded species (2) plant species with low RGRs are more tolerant of hydrocarbon-contaminated soil than those with high RGRs and (3) RGR and seed mass are correlated.

5.3 Materials and Methods

Crude oil-contaminated surface flare pit soil and uncontaminated soil from Carlyle, SK were collected in October, 2001 from a 0-20 cm depth using a shovel. The soil was transported to the lab in sealed one gallon plastic bins and sieved while still field moist to pass a 5-mm screen. Large rocks were removed and any soil clumps pushed through the sieve. Three parts contaminated soil was mixed with one part commercial potting soil (wt/wt) (Pamper Your Plant, Early's Farm and Garden Centre, Saskatoon, SK) as the seedlings either did not emerge or died shortly after emergence in unamended soil.

Soils were analyzed for percentage total petroleum hydrocarbons (EPA Method 3540A, Soxhlet extraction), pH (McLean, 1982) and electrical conductivity (EC) (Rhoades, 1982) using a 1:2 soil to solution ratio (Envirotest Laboratories, Saskatoon, SK) (Table 5.1). All soils were analyzed for pH (McLean, 1982) and electrical conductivity (EC) (Rhoades, 1982) using a 1:2 soil to solution ratio, and percentage

organic matter (% OM) by loss on ignition (Nelson and Sommers, 1982) (Envirotest Laboratories, Saskatoon, SK). Available orthophosphate P was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994) (Envirotest Laboratories, Saskatoon, SK). The orthophosphate ion reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex was reduced with ascorbic acid to form a blue complex, which was measured colorimetrically by auto analysis at 880 nm. Available potassium was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994) (Envirotest Laboratories, Saskatoon, SK). The extract was mixed with lithium nitrate, nitric acid and lanthanum oxide as an internal standard and passed into the burner of a flame photometer. Intensity of light emitted was measured at 768 nm. After organic matter oxidation with 1N H_2O_2, soil texture was determined using the pipette method (Gee and Bauder, 1986) (Envirotest Laboratories, Saskatoon, SK). Total carbon (%C) and nitrogen (%N) content of the soils were determined using a LECO CNS-2000 Analyzer (LECO Corporation, Mississauga, ON).

Table 5.1. Selected characteristics of uncontaminated and contaminated soils used to determine the effect of seed size and relative growth rate on plant performance.

Soils	Texture	TPH [a] (ppm)	pH	EC (mS/cm)	C (%)	N (%)	P (µg/g)	K (µg/g)	C:N
Uncontaminated	Clay	0	7.5	2.9	3.0	0.2	4.0	196	15:1
Contaminated [b]	Clay	14000	7.4	5.8	8.2	0.3	2.2	264	27:1

[a] Total Petroleum Hydrocarbons
[b] Amended with 1 part potting soil (Pamper Your Plant) to 3 parts contaminated soil

Eight plant species (Table 5.2) with seed masses spanning four orders of magnitude were obtained from a local seed supplier (Early's Farm and Garden Centre, Saskatoon, SK) and a local native seed grower (Prairie Mountain Seeds, Arcola, SK). Seed mass was determined by weighing 100 seeds individually for the two highest seed size classes or in lots of ten for the two lowest classes as the scale used would not register weights less than 0.099 mg. Prior to planting, Indian breadroot (*Psoralea esculenta* Pursh) and wild peavine (*Lathyrus venosus* Muhl) seeds were scarified by rubbing them between two sheets of sandpaper for one minute and soaking in 90% ethanol for one minute to inhibit fungal infection (Caetano et al., 1990). Seeds were incubated in the dark at room temperature on moistened filter paper in Petri dishes.

Table 5.2. Seed size class, seed mass, family and status of species included in the experiment. Values represent the mean±SE (n=70).

Seed Size Class	Seed Mass (mg)	Species	Family	Status [a]
>10 mg	28.622±3.015	*Psoralea esculenta* Pursh	Fabaceae	Native
	18.771±3.937	*Lathyrus venosus* Muhl.	Fabaceae	Native
9.9-1.0 mg	2.674±0.378	*Agropyron pectiniforme* R. and S.	Poaceae	Non-native
	2.572±0.313	*Melilotus officinalis* (L.) Lam.	Fabaceae	Non-native
0.9-0.1 mg	0.683±0.023	*Trifolium repens* L.	Fabaceae	Non-native
	0.442±0.029	*Phleum pratense* L.	Poaceae	Non-native
<0.1 mg	0.096±0.009	*Artemisia frigida* Willd.	Asteraceae	Native
	0.093±0.009	*Potentilla pensylvanica* L.	Rosaceae	Native

[a] Native species grew naturally in western Canada prior to European colonization while non-native species arrived after.

Germinants were transferred to 4-cm diameter, 20-cm long cone-tainers (Stuewe and Sons, Corvallis, OR) containing 120-g of amended contaminated or uncontaminated field soil. The experimental design was completely randomized with 35 replicates. The cone-tainers were placed in a Conviron (Winnipeg, MB) growth chamber receiving 16 hours light at 25 °C (±0.5 °C) and 8 hours dark at 15 °C (±0.5 °C). Lighting was provided by moveable banks of fluorescent and incandescent lights providing approximately 50,000 lux. These conditions were chosen to approximate optimal temperature and light conditions in Saskatchewan in summer.

The container capacity of each soil was determined (Cassel and Nielsen, 1986). Soils were kept at 60% of container capacity by watering daily to a predetermined weight. Blake (1935) indicated that one-half to two-thirds saturation was optimal for germination of native prairie plants.

Aerial and root biomass were harvested four weeks emergence. The shoot was cut off at the soil surface. Roots were removed by gently shaking the soil off and picking up any broken pieces with forceps. The roots were washed with distilled water to remove any clinging soil particles. Both roots and shoots were dried in an oven at 60 °C for 24-26 hours, and weighed. RGR was calculated using the following formula (Fitter and Hay, 1987):

$$RGR = \frac{\ln W_2 - \ln W_1}{t} \qquad [5.1]$$

Where W_2 = mean oven dry seedling mass at harvest, W_1 = mean oven dry initial seedling mass and t = length of the growing time (4 weeks). Initial seedling mass was determined by harvesting seedlings (n=35) 12 hours after germination on moist filter paper in Petri dishes and oven drying at 60 °C for 24 hours.

Student's t-tests between uncontaminated and contaminated seedling mass and RGR were conducted. The correlation between seed mass and seedling mass, seedling mass and RGR, and seed mass and RGR in both soils was determined. Logarithmic transformations to homogenize variances were applied to seedling biomass. All statistical tests were done using MINITAB software (MINITAB Inc., State College, PA) and a $P \leq 0.05$.

5.4 Results

Figure 5.1 shows the mean seedling mass of each species in uncontaminated and contaminated soil. Seedling mass in contaminated soil was significantly lower than in uncontaminated soil for all species. However, the percentage reduction of seedling mass in contaminated soil was different among species. The species with the largest seed, Indian breadroot, had a seedling mass reduction of less than 25% while the two species with the smallest seeds, pasture sage (*Artemisia frigida* L.) and prairie cinquefoil (*Potentilla pensylvanica* L.) had seedling mass reductions of almost 95%. There was a strong positive correlation between seed mass and seedling mass at day 28 in both contaminated and uncontaminated soil (Figure 5.2). However, the correlation between seed mass and seedling mass was stronger in the contaminated (r=0.976) than the uncontaminated soil (r=0.861).

The RGR of all species decreased in the contaminated soil compared to uncontaminated soil (Figure 5.3). The relative growth rate reduction was more than 72% for the two species with the smallest seeds, pasture sage and prairie cinquefoil, but only 32% for the largest seeded species, Indian breadroot. There was a strong negative

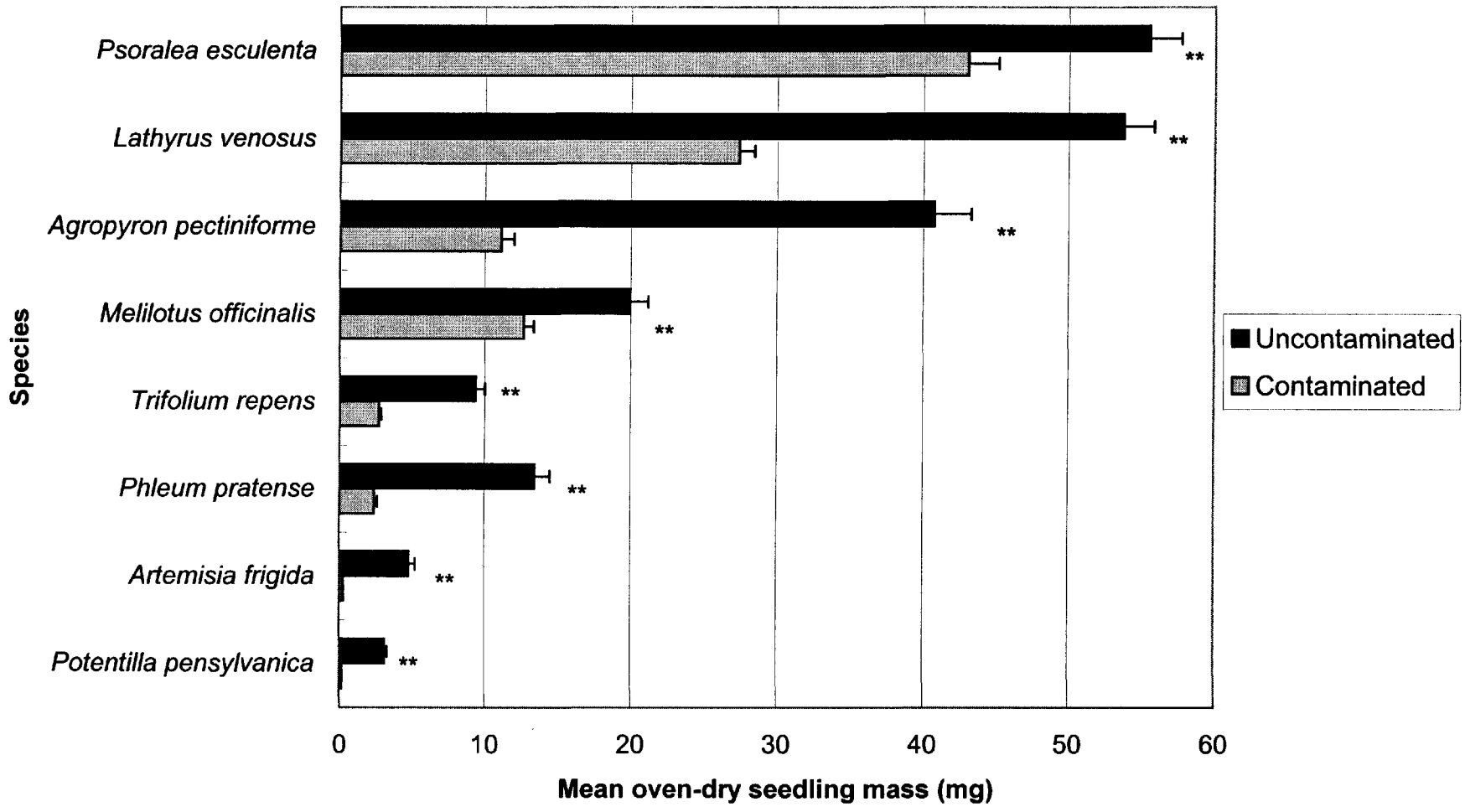

Figure 5.1. Mean oven-dry seedling mass of eight species 28 days after emergence in uncontaminated and crude-oil contaminated field soil. Ordered from the largest *(P. esculenta*) to the smallest (*P. pensylvanica*) seed mass. Statistically significant ($P \leq 0.001$) differences in values within species using Student's t-test denoted by **. Error bars represent +SE (n=35).

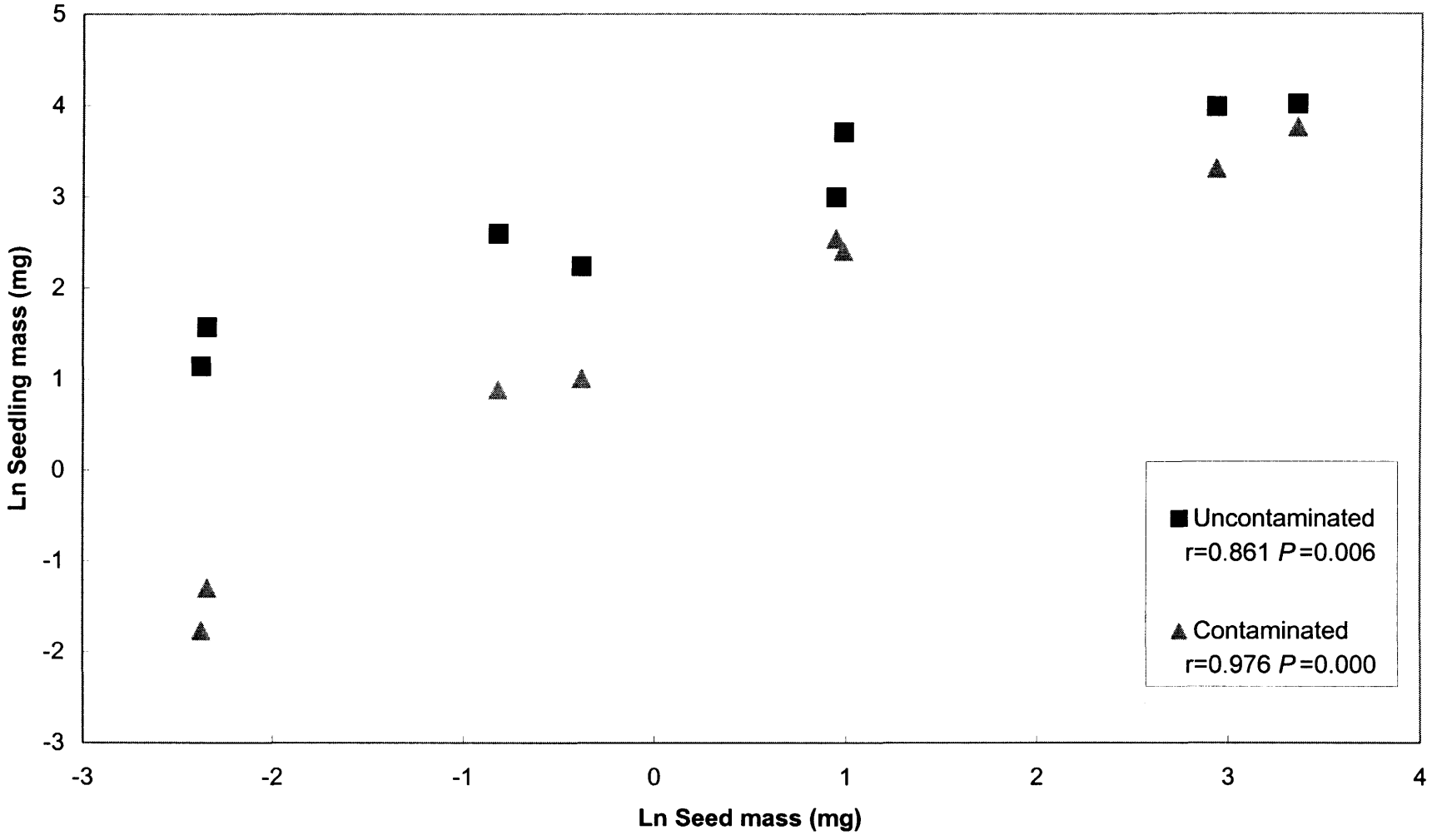

Figure 5.2. Relationship between seed mass and seedling mass 28 days after emergence in uncontaminated and crude oil-contaminated field soil.

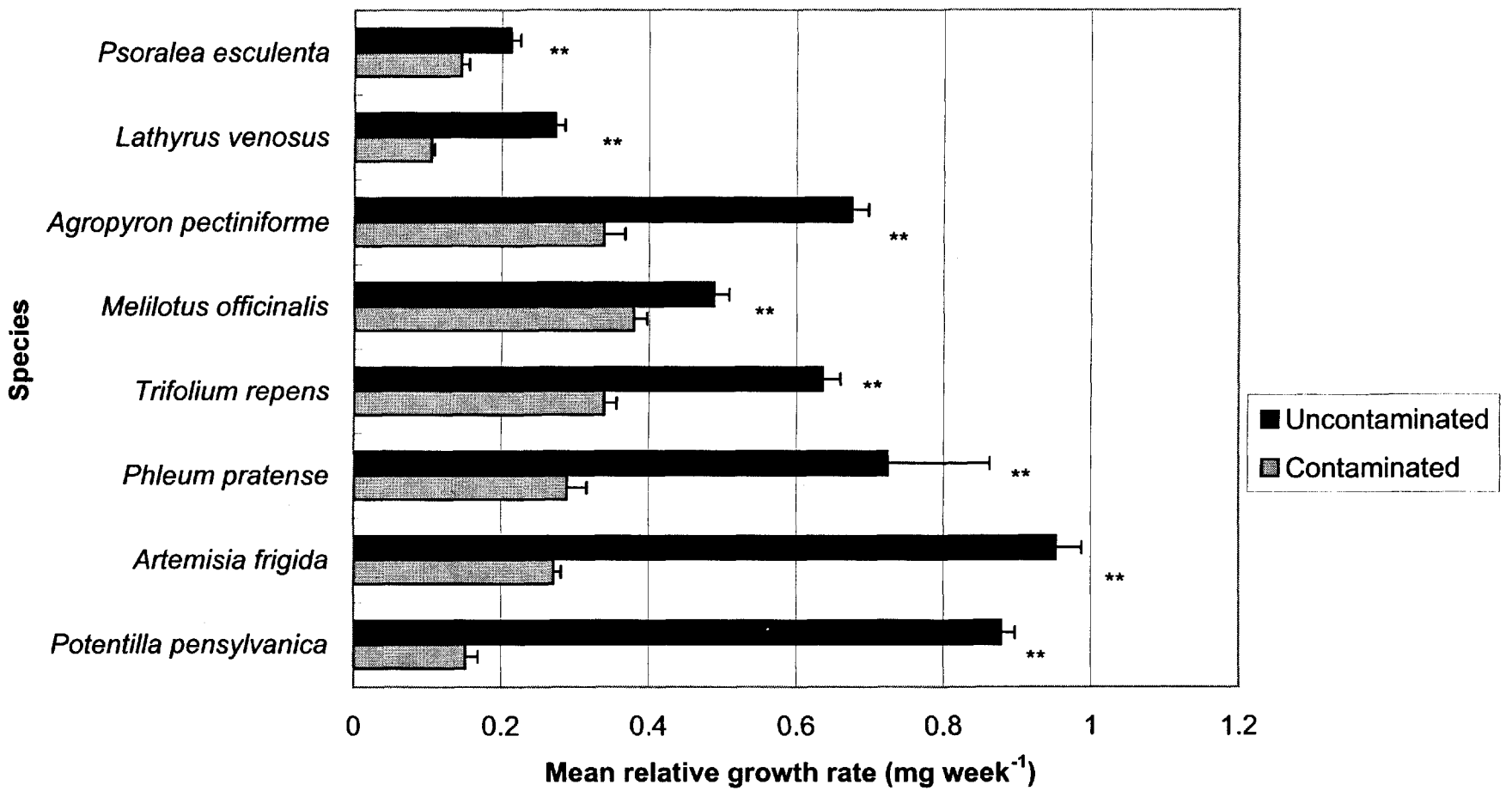

Figure 5.3. Mean relative growth rate of eight plants 28 days after emergence in uncontaminated and crude-oil contaminated field soil. Ordered from the largest (*P. esculenta*) to the smallest (*P. pensylvanica*) seed mass. Statistically significant ($P \leq 0.001$) differences in values within species using Student's t-test denoted by **. Error bars represent + SE (n=35).

correlation between the RGR in uncontaminated soil and seedling mass in both contaminated and uncontaminated soil (Figure 5.4). The correlation between RGR and seedling mass was stronger in the contaminated (r=-0.916) than in the uncontaminated soil (r=-0.860).

The relationship between seed mass and RGR in uncontaminated and contaminated soil is shown in Figure 5.5. These results indicate that seed mass and RGR in uncontaminated soil are negatively correlated (i.e. species with large seeds tended to have low initial growth rate). However, the correlation between seed mass and RGR in contaminated soil was not significant at $P \leq 0.05$. The lack of a significant correlation between seed mass and RGR in contaminated soil was due to the negative effect the contaminated soil had on RGR, particularly on small seeded species.

5.5 Discussion

To understand the results of this study, how the presence of hydrocarbons in soil affects plant growth must be considered. Directly, hydrocarbons reduce plant growth due to its phytotoxic properties (Chaîneau et al., 1997). As crude oil comprised 14,000 ppm (wt/wt) of the contaminated soil in this study, poor plant growth may be due to toxic effects of the oil. Indirectly, hydrocarbons influence nutrient availability (Udo and Fayemi, 1975, Abujnah, 1999) and salinity (Stahl and Williams, 1986). Although the addition of hydrocarbons to soil increases total nitrogen, available nitrogen is typically lower due to microbial immobilization resulting from the elevated C:N ratio of the material (Toogood, 1977; Xu and Johnson, 1997). At C:N ratios less than 20:1 mineralization occurs, at ratios greater than 30:1 immobilization occurs and between

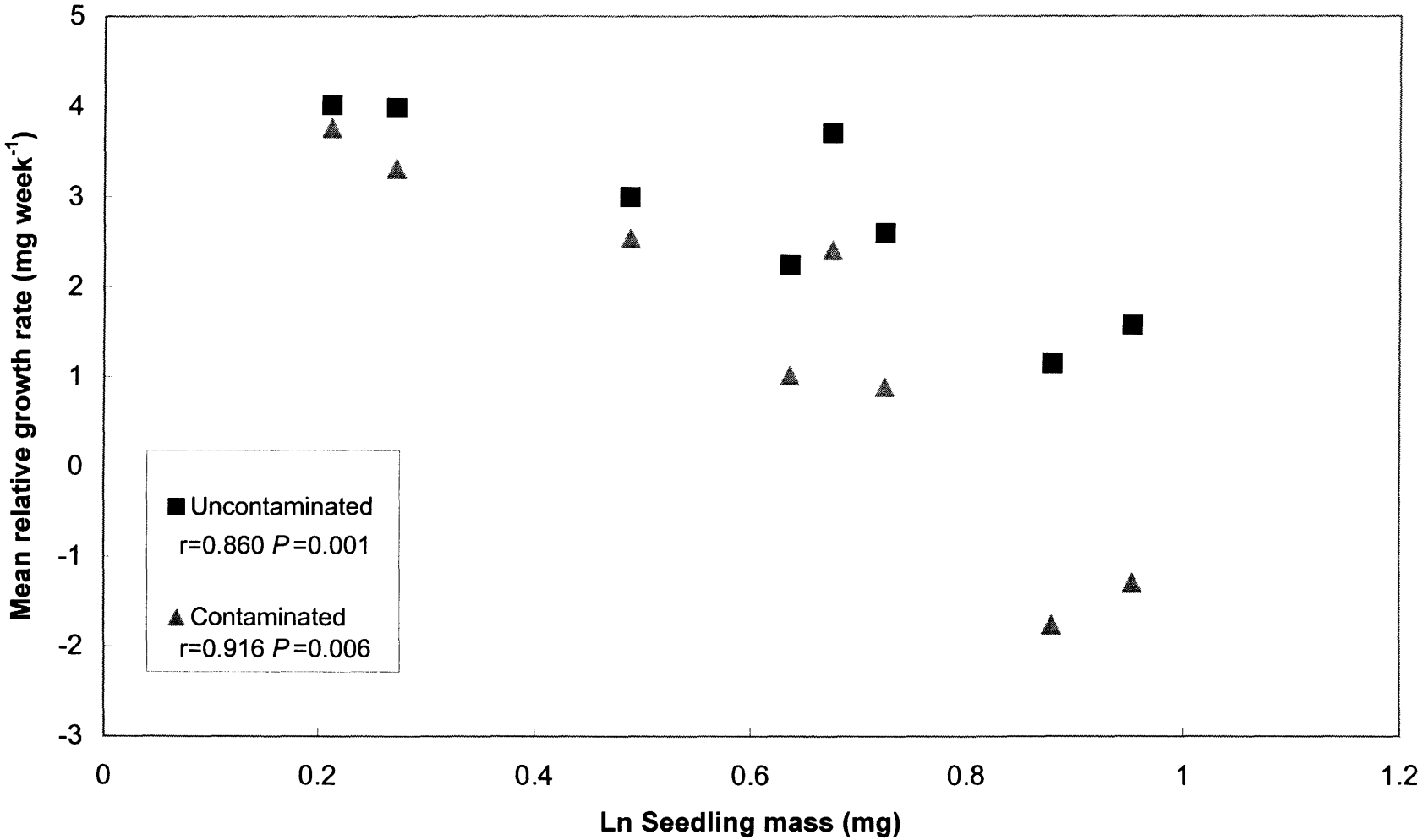

Figure 5.4. Relationship between relative growth rate in uncontaminated soil and seedling mass 28 days after emergence in uncontaminated and crude oil-contaminated field soil.

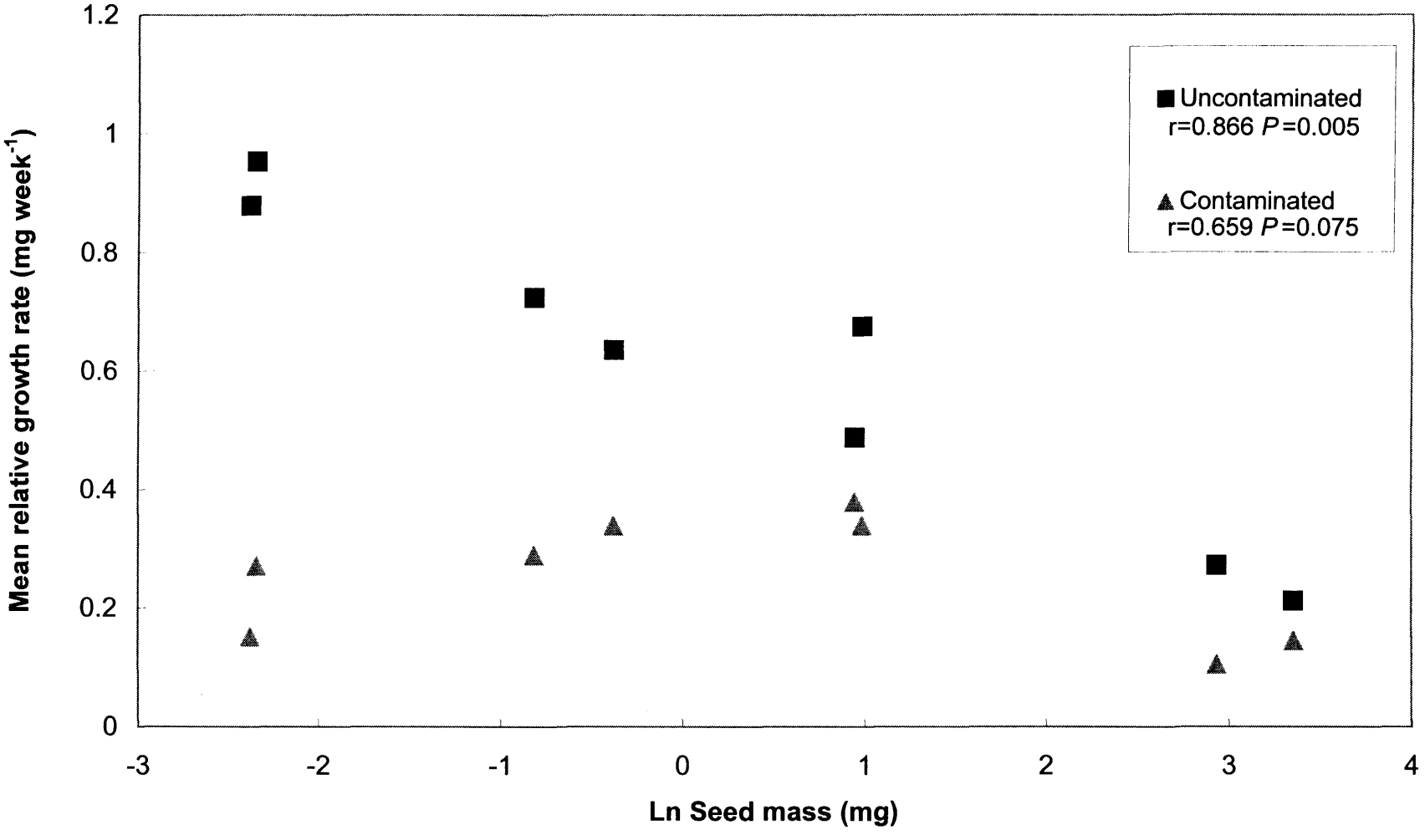

Figure 5.5. Relationship between seed mass and relative growth rate 28 days after emergence in uncontaminated and crude oil - contaminated field soil.

20:1 and 30:1 immobilization and mineralization are approximately equal (Tisdale et al., 1993).

The C:N ratio in the uncontaminated soil was 15:1 and in the contaminated 27:1, suggesting that nitrogen availability was higher in the uncontaminated soil because mineralization would be occurring. Total phosphorus was slightly lower in contaminated than uncontaminated soil, which may have negatively affected plant growth. Furthermore, Udo and Fayemi (1975) found that extractable P decreases with hydrocarbon addition, so P may have been less available in the contaminated soil. Hydrocarbons may also affect nutrient availability by coating plant roots and interfering with normal uptake (Udo and Fayemi, 1975).

Salinity may have also affected plant growth, as the EC of the contaminated soil was higher than the uncontaminated soil. Salt sensitive plants cannot tolerate an EC >1.3 mS/cm and moderately sensitive plants cannot tolerate an EC >3.0 mS/cm (Maas, 1990). In the uncontaminated soil the EC was 2.9 mS/cm so according to Maas (1990) we could expect sensitive plants to experience a yield loss of around 50%. In the contaminated soil the EC was 5.8 mS/cm so sensitive plants would experience yield losses around 75% and moderately sensitive plants between 25% and 50% (Maas, 1990). Timothy (*Phleum pratense* L.) and white clover (*Trifolium repens* L.) are classified as moderately sensitive (Maas, 1990) so some of their yield loss could be due to soil salinity. Yellow sweet-clover (*Melilotus officinalis* L.) is moderately salt tolerant and crested wheatgrass is tolerant so their yield would not have been affected by EC, unless their seedlings were sensitive (Maas, 1990). The salt sensitivity of the remaining

four plants (all native to western Canada) has not been determined, but they are not typically found in saline habitats in the wild, suggesting some sensitivity.

When grown for 28 days in hydrocarbon-contaminated and uncontaminated soil, total seedling mass of the eight species studied was correlated with seed mass. This is because a large seed can produce a larger seedling than a small seed as it has greater nutrient reserves (Milberg et al., 1998). The advantage of a large seed is typically lost after 15-20 days (Jurado and Westoby, 1992) as plants with smaller seeds have higher RGR and will eventually catch up (Swanborough and Westoby, 1996). Since the plants in this study were only 28-days old, large seed mass was still providing an advantage to some seedlings, resulting in a correlation between seed mass and seedling mass in both uncontaminated and contaminated soil. However, large seed mass may remain an advantage for a longer time under nutrient deficient conditions (Poorter and Garnier, 1999). A large seedling has a better chance of intercepting scarce nutrients than a small seedling, due to a larger initial root system (Lloret et al., 1999). Several researchers found a stronger correlation between seed mass and seedling mass under nutrient-deficient conditions compared to a control (Atkinson, 1973; Kolawole and Kang, 1997; Milberg et al., 1998). This pattern was also observed in this study with the correlation between seed mass and seedling mass being stronger in contaminated than uncontaminated soil.

Seed mass alone does not explain the results of this study. Although crested wheatgrass had the third largest seeds (2.674 mg), its seedling mass was reduced by 72%, while yellow sweet-clover, a species with similar sized seeds (2.572 mg), had a mass reduction of only 36%. The differences in mass reduction of these two species

may be explained by differences in the RGR. The RGR of crested wheatgrass in uncontaminated soil was 0.68 mg $week^{-1}$ but only 0.49 mg $week^{-1}$ in yellow sweet-clover. White clover, a species with a similar RGR as crested wheatgrass in uncontaminated soil (about 0.65 mg $week^{-1}$), had a similar seedling mass reduction in contaminated soil, about 71%. Thus it appears that both seed mass and RGR are responsible for the results observed.

Seedling mass and RGR were highly correlated in this study, more so when grown in contaminated than uncontaminated soil. This result is not unexpected, as others have documented a correlation between RGR and seedling mass (Fenner, 1983; van der Werf et al., 1993). A low RGR is one of the traits that enable plants to grow in low resource habitats in the wild (Grime, 1979; Chapin et al., 1993). Plants with low RGR experience fewer losses of biomass due to leaf turnover, which is advantageous in a nutrient-poor environment (Poorter and Garnier, 1999). Slow growth also means lower rates of photosynthesis and thus less water loss, which confers stress resistance in dry and saline habitats (Chapin et al., 1993). Slow growth also indirectly confers stress resistance by reducing carbon demands for growth, allowing greater carbon allocation to processes that directly contribute to stress resistance like nutrient storage, chemical defense or detoxification (Chapin et al., 1993). Lambers and Poorter (1993) hypothesize that root exudation is more important in slow growing species from nutrient-poor habitats than in fast growing species from nutrient-rich habitats. The production of root exudates like siderophores (Römheld and Marschner, 1986), chelating compounds (Römheld, 1987) and organic acids (Hoffland et al., 1989) facilitate acquisition of nutrients that are otherwise unavailable.

Ecologists have determined that as a general rule seed mass and RGR are negatively correlated (Fenner and Kitajima, 1999). The results of this study concur but only when plants are grown in uncontaminated soil. In hydrocarbon-contaminated soil the RGR of small seeded species was greatly reduced, which decreased the correlation. The functional basis of the relationship between seed mass and RGR is probably via correlation of seed mass with patterns of biomass allocation rather than with metabolic or photosynthetic rates (Fenner and Kitajima, 1999). One of the components of RGR is specific leaf area (SLA; leaf area divided by leaf mass) (Poorter and Garnier, 1999). Plants with low SLA tend to have longer leaf life spans due to investment in structures that decrease herbivory, water loss, nutrient leaching and cold resistance, which increases nutrient use efficiency (Poorter and Garnier, 1999). Consequently species with low SLA have less leaf area devoted to photosynthesis. Large-seeded species tend to have lower SLA, and thus a lower RGR (Poorter and Garnier, 1999).

It is impossible to say exactly which environmental factors are having the greatest effect on biomass production in contaminated soil. The reason for the strong correlation between seed mass, RGR and seedling mass may be precisely because there are multiple stressors in hydrocarbon-contaminated soil. When plants are exposed to only a single source of stress, seed mass is not always correlated with RGR (Chapin et al., 1989; Stock et al., 1990) or seedling mass (Mazer, 1989). Thus it could be a combination of hydrocarbon toxicity, low nutrient availability and salinity that is causing the stronger correlation between seed mass, RGR and seedling mass in contaminated soil.

5.6 Conclusions

The seedling biomass and RGR of all eight species was significantly lower in hydrocarbon-contaminated compared to uncontaminated soil. The hypotheses that species with large seeds and low RGR will be more tolerant of hydrocarbon-contaminated soil than species with small seeds and high RGR was supported by this research. Furthermore correlations were stronger when species were grown in hydrocarbon-contaminated soils. A significant correlation between seed mass and RGR in uncontaminated soil was noted. Additional testing of these hypotheses using a wider variety of species is needed to determine if this is a general pattern among all plant species.

6.0 DEGRADATION OF HYDROCARBONS BY TWO TOLERANT PLANT SPECIES IN CONTAMINATED FIELD SOIL

6.1 Abstract

The objective of this research was to determine if two reportedly hydrocarbon-tolerant species, Indian breadroot (*Psoralea esculenta* Pursh) and crested wheatgrass (*Agropyron pectiniforme* R. & S.) were capable of accelerating the degradation of hydrocarbons in field soil. The species were grown singly and together in pots to evaluate biomass production and hydrocarbon degradation. Growing crested wheatgrass and Indian breadroot plants together increased the biomass production of the former compared to the treatment where only crested wheatgrass plants were grown, likely due to low interspecific competition.

The concentrations of seven known polycyclic aromatic hydrocarbons (PAHs) were not affected by the presence of plants. However, the overall hydrocarbon profile as shown in gas chromatograms was smaller in planted compared to unplanted treatments. The crested wheatgrass treatment had the smallest hydrocarbon profile, followed by the mixed and Indian breadroot treatments. This suggests crested wheatgrass is a better phytoremediator, at least in the seedling stage.

6.2 Introduction

Hydrocarbon contamination of soil is a growing problem due to the increased extraction of oil and gas, which inevitably leads to accidental oil spills. As many hydrocarbons are carcinogenic, steps must be taken to remediated contaminated soil. Fortunately, unlike inorganic contaminants, most hydrocarbons can be degraded by microorganisms like bacteria (Boldrin et al., 1993; Brown et al., 1998), actinomycetes (Radwan et al., 1995) and fungi (Sutherland, 1992; Donnelly and Fletcher, 1994). Many studies show that growing plants in hydrocarbon-contaminated soil accelerates the degradation rate of hydrocarbons (Aprill and Sims, 1990; Reilley et al., 1996; Hutchinson et al., 2001b). Although the importance of various mechanisms is still uncertain, plants appear to increase degradation by improving the soil environment for hydrocarbon-degrading microorganisms. Some plants release compounds from their roots that act as cometabolites for microbial degradation of hydrocarbons (Ferro et al., 1997). Other plant species volatilize (Watkins et al., 1994; Kroening et al., 2001), store (Edwards et al., 1982; Wild and Jones, 1992) or degrade hydrocarbons (Edwards et al., 1982; Edwards, 1988). Plants generally improve oxygen availability and soil structure (Cunningham et al., 1996; Hutchinson et al., 2001b).

Not all studies find significant reductions in total petroleum hydrocarbons when plants are grown in contaminated soil (Ferro et al., 1994; Ferro et al., 1997; Kulakow et al., 2000). However, even though the total concentration of petroleum hydrocarbons does not decrease, the concentrations of some hydrocarbons may decline. The degradation pathway of a four aromatic ring carbon compound like pyrene first involves decomposition into a three-ring hydrocarbon, then a two-ring hydrocarbon and so on.

By using a technique like solid-phase microextraction (SPME) changes in the hydrocarbon profile can be detected (Havenga and Rohwer, 1999).

Some research indicates that growing more than one species of plant together benefits productivity. For example, growing clover and grass together under field conditions improves the growth of grass, probably due to the fixation of nitrogen by the clover (Ta and Faris, 1987). Aprill and Sims (1990) note that hydrocarbons are degraded when a mixture of eight prairie grasses is grown in contaminated soil. Unfortunately, the effect that each individual plant species had on degradation is not recorded. Could there be a synergy occurring, where the degradation by a mixture of plant species is greater than the sum of individual plant species degradation? Or is the opposite true? These questions still need to be answered.

The purpose of this experiment was to determine if two reportedly hydrocarbon tolerant species, crested wheatgrass and Indian breadroot accelerated hydrocarbon degradation in weathered contaminated field soil for the first 16 weeks of growth. The effect that planting the two species together had on plant growth and degradation was also examined. A secondary objective was to evaluate the usefulness of SPME.

6.3 Materials and Methods

6.3.1 Soil and plant preparation

Crude oil-contaminated surface flare pit soil (120 kg) from a site near Hardisty, AB was collected in July, 2001 from a 0-20 cm depth using a shovel and brought back to the lab under room temperature conditions in sealed 1 gallon plastic buckets. The soil was air dried to 17% moisture the day after it was obtained and sieved to pass a 5-

mm screen. During the sieving process small rocks, twine, clumps of tar and root mats were removed and the soil mixed by hand to ensure homogeneity.

Soils were analyzed for percentage total petroleum hydrocarbons (EPA Method 3540A, Soxhlet extraction), pH (McLean, 1982) and electrical conductivity (EC) (Rhoades, 1982) using a 1:2 soil to solution ratio. Available nitrate (NO_3-N) was extracted from the soil using a dilute (0.001 N) calcium chloride solution (Martin, 1993). Nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye had magenta color which was measured at colorimetrically at 520nm. Available orthophosphate P was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994). The orthophosphate ion reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex was reduced with ascorbic acid to form a blue complex, which was measured colorimetrically by auto analysis at 880 nm. Available potassium was extracted from the soil using Modified Kelowna extracting solution (0.025M HOAc, 0.25M NH4Oac, 0.015M NH4F at pH 4.5) (Qian et al., 1994). The extract was mixed with lithium nitrate, nitric acid and lanthanum oxide as an internal standard and passed into the burner of a flame photometer. Intensity of light emitted was measured at 768 nm. After organic matter oxidation with 1N H_2O_2 soil texture was determined using the pipette method (Gee and Bauder, 1986). Envirotest Laboratories (Saskatoon, SK) conducted all soil analyses.

Eleven-month old Indian breadroot seed was obtained from a local native seed grower (Prairie Mountain Seeds, Arcola, SK) and ten-month old crested wheatgrass seed from a local seed supplier (Early's Farm and Garden Centre, Saskatoon, SK). Prior to planting, Indian breadroot seeds were scarified by rubbing between two sheets of sandpaper for about one minute, and soaking in 90% ethanol for one minute to inhibit fungal infection (Pahl and Smreciu, 1999; Caetano et al., 1990).

6.3.2 Experimental design

There were four treatments: unplanted, Indian breadroot, crested wheatgrass and a mixed species treatment with both Indian breadroot and crested wheatgrass. The pure live seed of both Indian breadroot and crested wheatgrass was 95%. The experimental design was completely randomized with eight replicates. In the single species treatments eight seeds were planted in 10-cm diameter, 40-cm long PVC tubes covered with four layers of cheesecloth on the bottom. Each tube contained 3.7-kg of contaminated soil. In the mixed species treatment four seeds each of crested wheatgrass and Indian breadroot were sown. The seedlings were thinned to four plants per tube (two plants of each species in the mixed tubes), except in the Indian breadroot treatment where only three seeds per tube survived to the end of the experiment. The PVC tubes were placed in a Conviron (Winnipeg, MB) growth chamber receiving 16 hours light at 25° C (±0.5° C) and 8 hours dark at 15° C (±0.5° C).

Lighting was provided by moveable banks of fluorescent and incandescent lights providing approximately 50,000 lux. These conditions were chosen to approximate optimal temperature and light conditions in Saskatchewan in summer. The container

capacity of each soil was determined (Cassel and Nielsen, 1986). Blake (1935) indicated that one-half to two-thirds saturation was optimal for germination of native prairie plants so soils were kept at 60% of container capacity by watering daily to a predetermined weight. Every four weeks the pots were fertilized with half strength Hoagland's solution (Went, 1957) to provide macro- and micronutrients for plant growth.

After 16 weeks shoots were cut at the soil surface. Roots were removed by gently shaking the soil off and picking up any broken pieces with forceps. The roots were then washed with distilled water to remove any clinging soil particles. Both roots and shoot were dried at 60 °C for 24-26 hours and weighed.

6.3.3 Soil analyses

Solid-phase microextraction (SPME) measures the volatile hydrocarbon compounds in the headspace that adsorb onto a polymer-coated fused silica fiber (Havenga and Rohwer, 1999). This method was chosen because it is a rapid, solventless technique that allows observation of changes in the volatile hydrocarbon profile, which may indicate degradation of some compounds (Havenga and Rohwer, 1999). Eight soil samples were analyzed prior to use in the growth chamber experiment. At the end of the experiment one soil sample from the top 0-5 cm of each unplanted tube and rhizosphere soil from planted tubes was collected for hydrocarbon analysis. Rhizosphere soil was collected by gently shaking the roots free of the bulk soil and collecting the soil that clung to the roots. For the mixed species treatment, rhizosphere soil from both species was collected and 0.5 g of each mixed together.

Approximately 1 g of bulk or rhizosphere soil from each replicate was placed in a sealed 25 mL glass vial and a needle containing a protractible fiber introduced into the 20 mL headspace. The vial was incubated at 30 °C for 45 minutes. After incubation the fiber was retracted and inserted into a gas chromatograph (GC) equipped with a flame ionization detector (Hewlett Packard Series II 5890). The quantity of hydrocarbon that adsorbed to the coating on the fiber is proportional to the volatile amount in the sample (Havenga and Rohwer, 1999). The GC profiles of all treatments were compared to the profiles of the original soil to determine if any changes had occurred.

To determine the concentration of eight known PAHs in the contaminated field soil, uncontaminated soil was mixed with 500, 750, 1,000, 2,500 or 5,000 ppm acenaphthene, acenaphthylene, anthracene, 2-bromonaphthalene, fluorene, naphthalene, phenanthrene and pyrene (Havenga and Rohwer, 1999). One gram of the soil/hydrocarbon mix was incubated at 30 °C for 45 minutes with the fiber, and the GC area values used to create calibration curves. An example of a calibration curve for acenaphthene is presented in figure 6.1. The equation and R^2 value for the other seven PAHs are in Table 6.1.

6.3.4 Statistical analyses

Biomass production between planted treatments was analyzed using Analysis of Variance (ANOVA) and Tukey's least significant difference (LSD). Mean biomass produced per plant between single and mixed species pots was analyzed using Student's t-tests. ANOVA and Tukey's LSD were used to compare the PAH concentrations

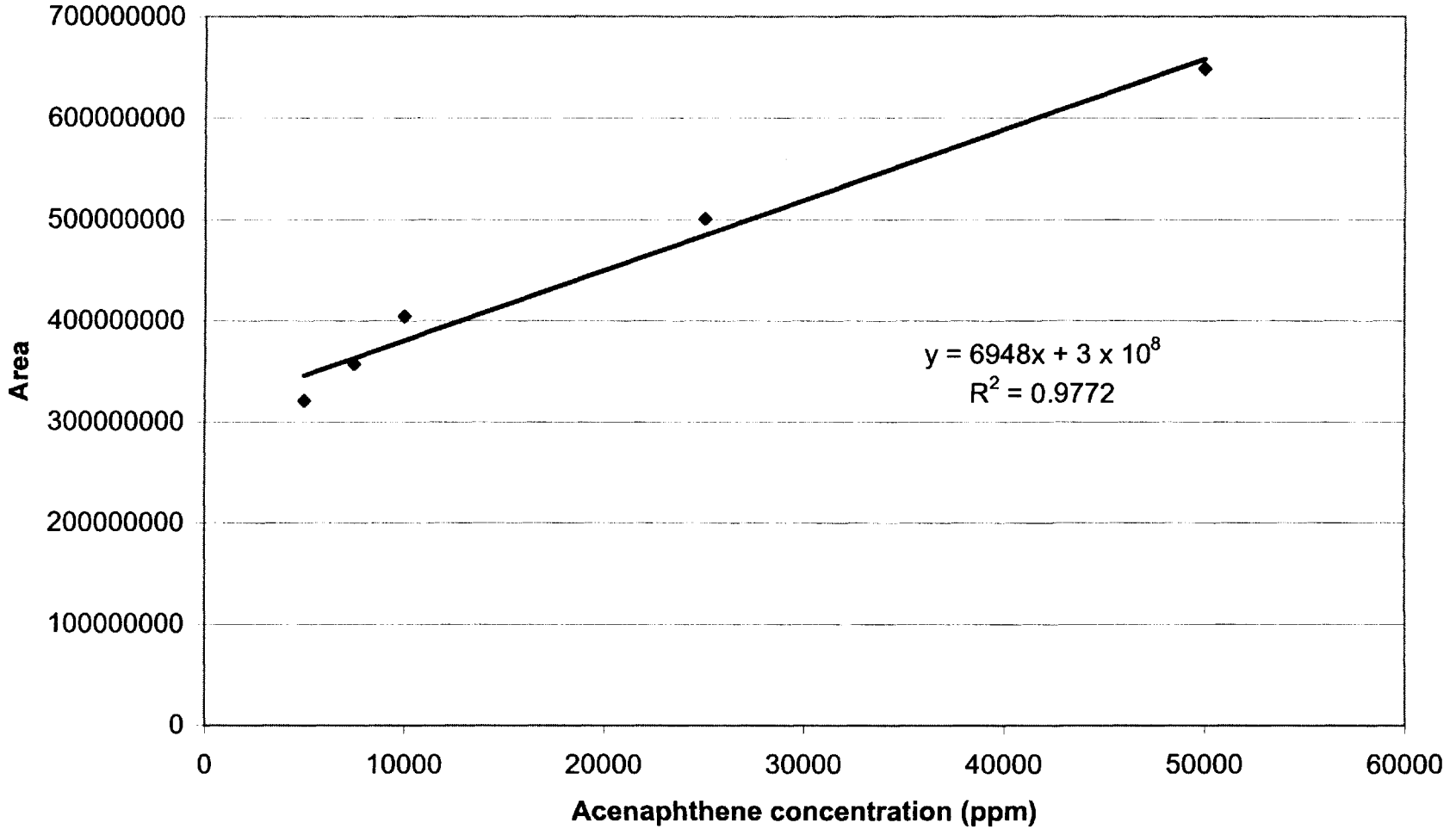

Figure 6.1. Calibration curve for acenaphthene using solid phase microextraction and a gas chromatograph.

between the four treatments. All statistical tests were done using MINITAB software (MINITAB Inc., State College, PA) and a $P \leq 0.05$.

Table 6.1 The equation and R^2 value from the calibration curves of seven polycyclic aromatic hydrocarbons.

Polycyclic Aromatic Hydrocarbon	Equation	R^2
2-bromonaphthalene	$y = 7596.7x + 3 \times 10^8$	0.9799
Acenaphthylene	$y = 7322.7x + 3 \times 10^8$	0.9852
Anthracene	$y = 7571.1x + 2 \times 10^8$	0.9802
Fluorene	$y = 6926.3x + 3 \times 10^8$	0.9768
Naphthalene	$y = 7278.6x + 3 \times 10^8$	0.9900
Phenanthrene	$y = 7158.3x + 2 \times 10^8$	0.9908
Pyrene	$y = 6997.8x + 2 \times 10^8$	0.9888

6.4 Results

6.4.1 Soil characteristics

The soil had a clay loam texture with 19,000 ppm (wt/wt) total petroleum hydrocarbons, a pH of 7.7, and an electrical conductivity of 0.3 mS/cm. Available nitrogen was 2.2 μg/mL, phosphorus 4.1 μg/mL, and potassium 55 μg/mL.

6.4.2 Biomass production

The crested wheatgrass treatments produced less total and root biomass than the Indian breadroot and mixed species treatments (Figure 6.2). Growth chamber studies

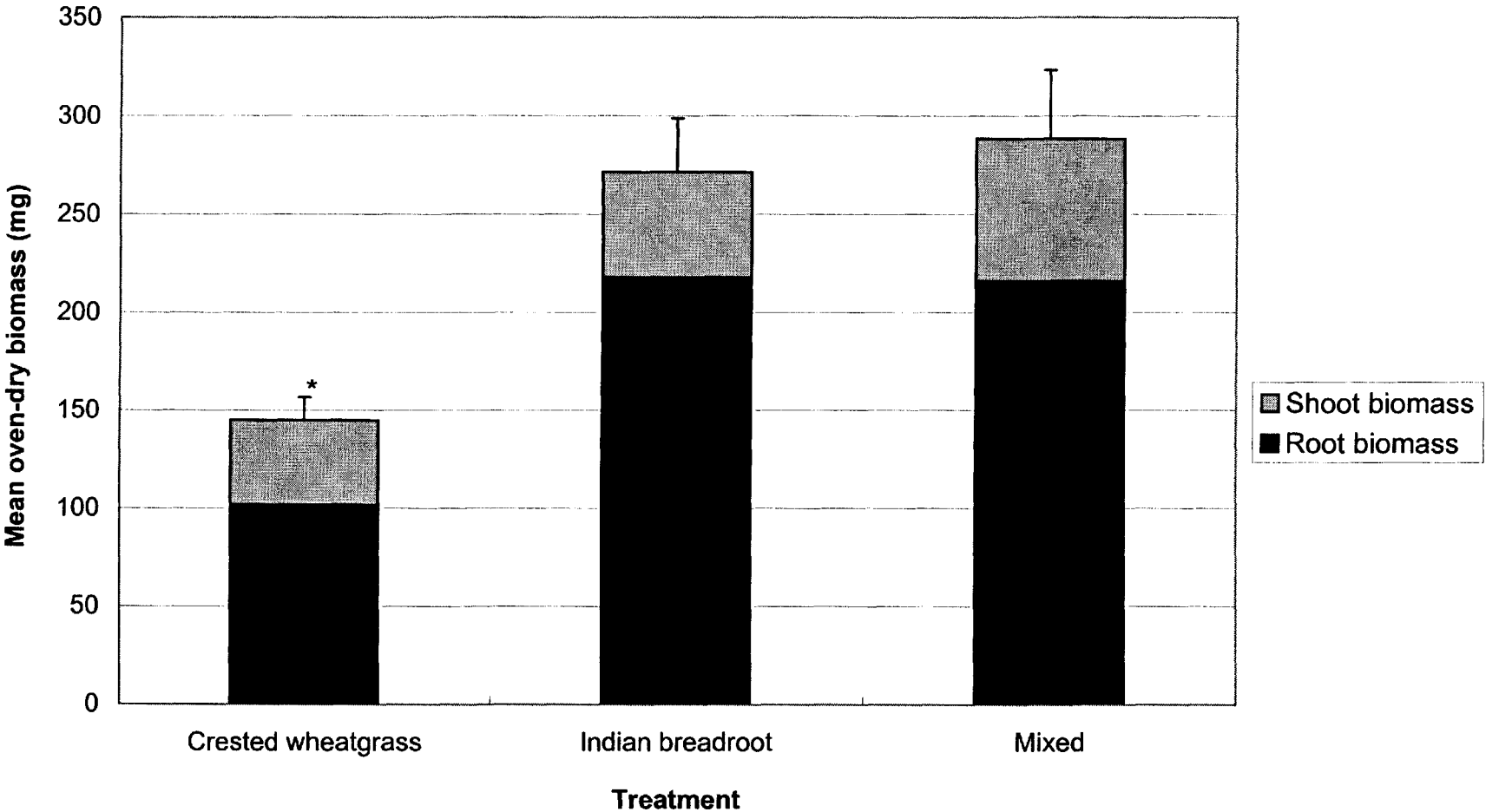

Figure 6.2. Mean oven-dry biomass production per planted treatment after 16 weeks in hydrocarbon-contaminated field soil. Statistically significant ($P \leq 0.05$) differences in values using ANOVA and Tukey's LSD denoted by *. Error bars represent + SE (n=8).

conducted on these species (chapters 4 and 5) suggest that total biomass of Indian breadroot would be higher than that produced by crested wheatgrass, although root biomass of the two species may be similar. The amount of total and root biomass produced in the Indian breadroot and mixed treatments were similar but the former might have been higher if four plants instead of three had survived per tube. Individual Indian breadroot plants produced about the same amount of total and root biomass whether grown in the single or mixed species treatment (Figure 6.3). In contrast, crested wheatgrass plants grown in the single species treatment produced significantly less total and root biomass per plant (about 40% less) than in the mixed species treatment.

6.4.3 Hydrocarbon degradation

The concentrations of 2-bromonaphthalene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene and pyrene did not significantly differ among the four treatments or the original soil (Figure 6.4). Acenaphthene concentration was significantly different among the treatments. The concentration of acenaphthene in the original soil, and the unplanted and Indian breadroot treatments was almost 400 ppm but around 550 ppm in the mixed and crested wheatgrass treatments. The volatility of the hydrocarbons in field soil was probably lower than that of pure hydrocarbons mixed with soil due to their gradual loss over time and adsorption to clay and organic matter. Therefore, the actual concentration of hydrocarbons in the field soil was likely higher. The hydrocarbon profile of the unplanted treatment was similar to the profile of

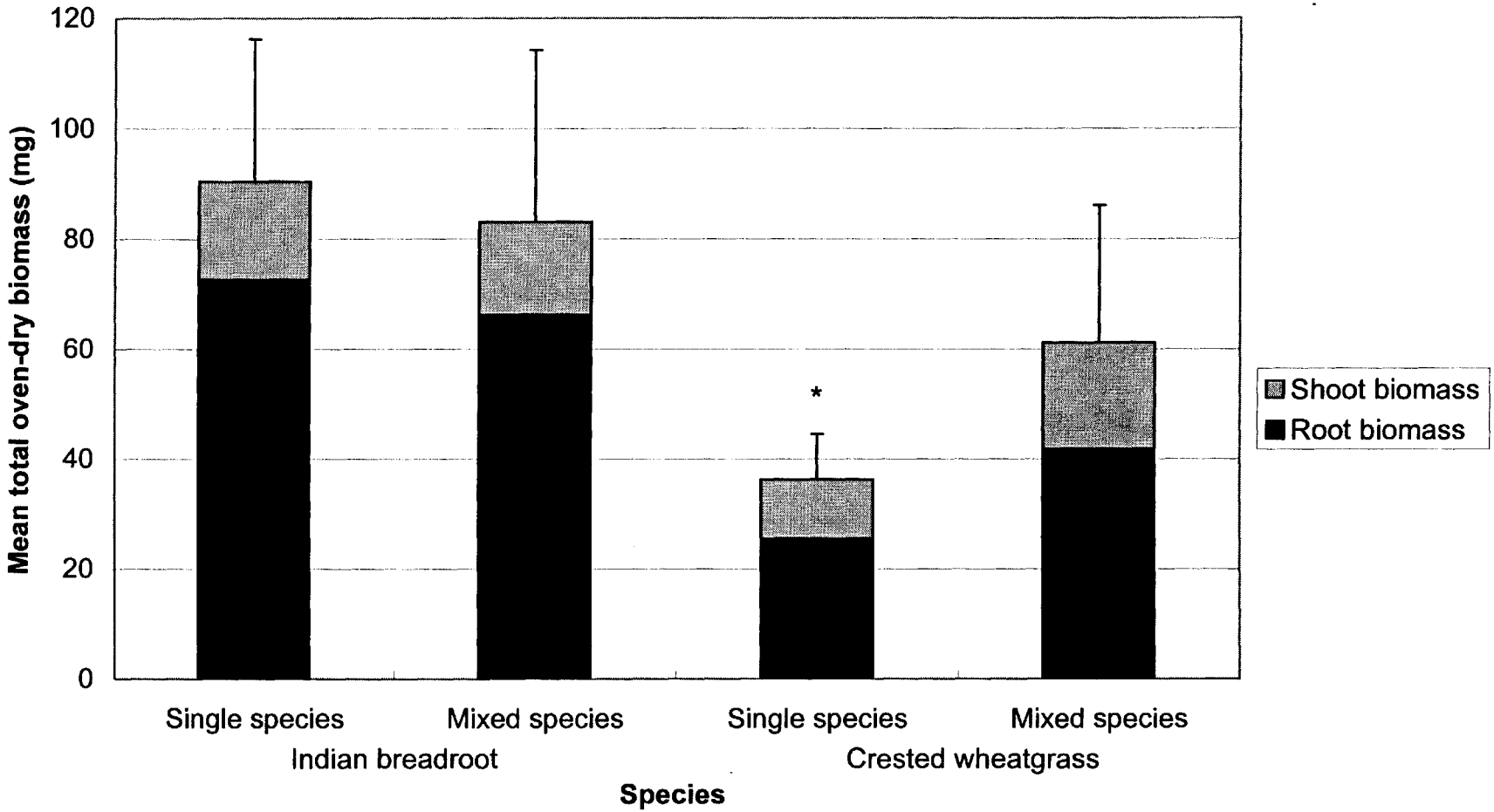

Figure 6.3. Mean oven-dry biomass produced per individual plant in single and mixed species treatments after 16 weeks growth in hydrocarbon-contaminated field soil. Statistically significant ($P \leq 0.05$) differences in values using Student's t-tests denoted by *. Error bars represent + SE (n=8).

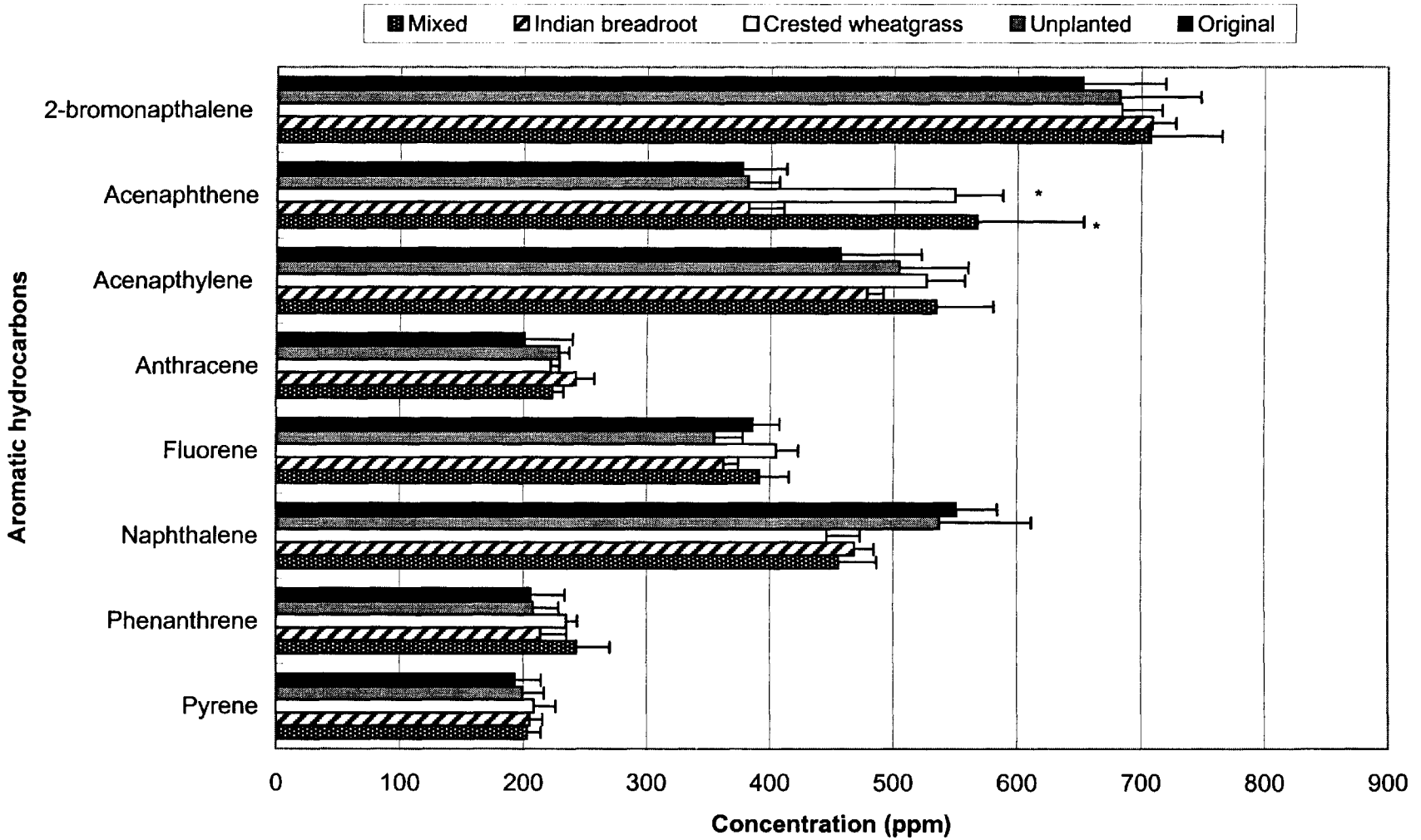

Figure 6.4. Concentration of eight aromatic hydrocarbons in soil after 16 weeks. Statistically significant ($P \leq 0.05$) differences in values compared to unplanted treatment using ANOVA and Tukey's LSD denoted by *. Error bars represent + SE (n=8).

the original soil, except near the beginning where hydrocarbons were present in smaller quantities in the unplanted treatment (Figure 6.5). In the Indian breadroot treatment (Figure 6.6) the quantity of hydrocarbons was lower than the original and unplanted treatment. The crested wheatgrass treatment (Figure 6.7) showed the greatest change in the hydrocarbon profile; it was considerably lower than the unplanted and Indian breadroot treatments. The mixed treatment (Figure 6.8) profile was intermediate between the Indian breadroot and crested wheatgrass treatment.

6.5 Discussion

This research suggests there may be a benefit to growing grasses with legumes in hydrocarbon-contaminated soil for the purposes of phytoremediation. In the mixed species treatment biomass production of individual crested wheatgrass plants was almost 40% higher than in the single species treatment. Vavrek and associates (2002) also found that individual plant yield was higher when different species were grown together in hydrocarbon-contaminated soil. When two or more species are grown together, intraspecific competition for nutrients is not as strong as in a monoculture, particularly if the two species have different root systems as no two species share the exact same niche (Fitter and Hay, 1997; Vavrek et al., 2002). Gerardo and associates (2001) note that competition between deep-rooted species and shallow-rooted species is half that of deep-rooted species competing with other deep-rooted species. When deep-rooted forbs are grown with grasses, the forbs tend to draw moisture and nutrients from lower in the soil horizon than the grasses (Coupland and Johnson, 1965). As crested

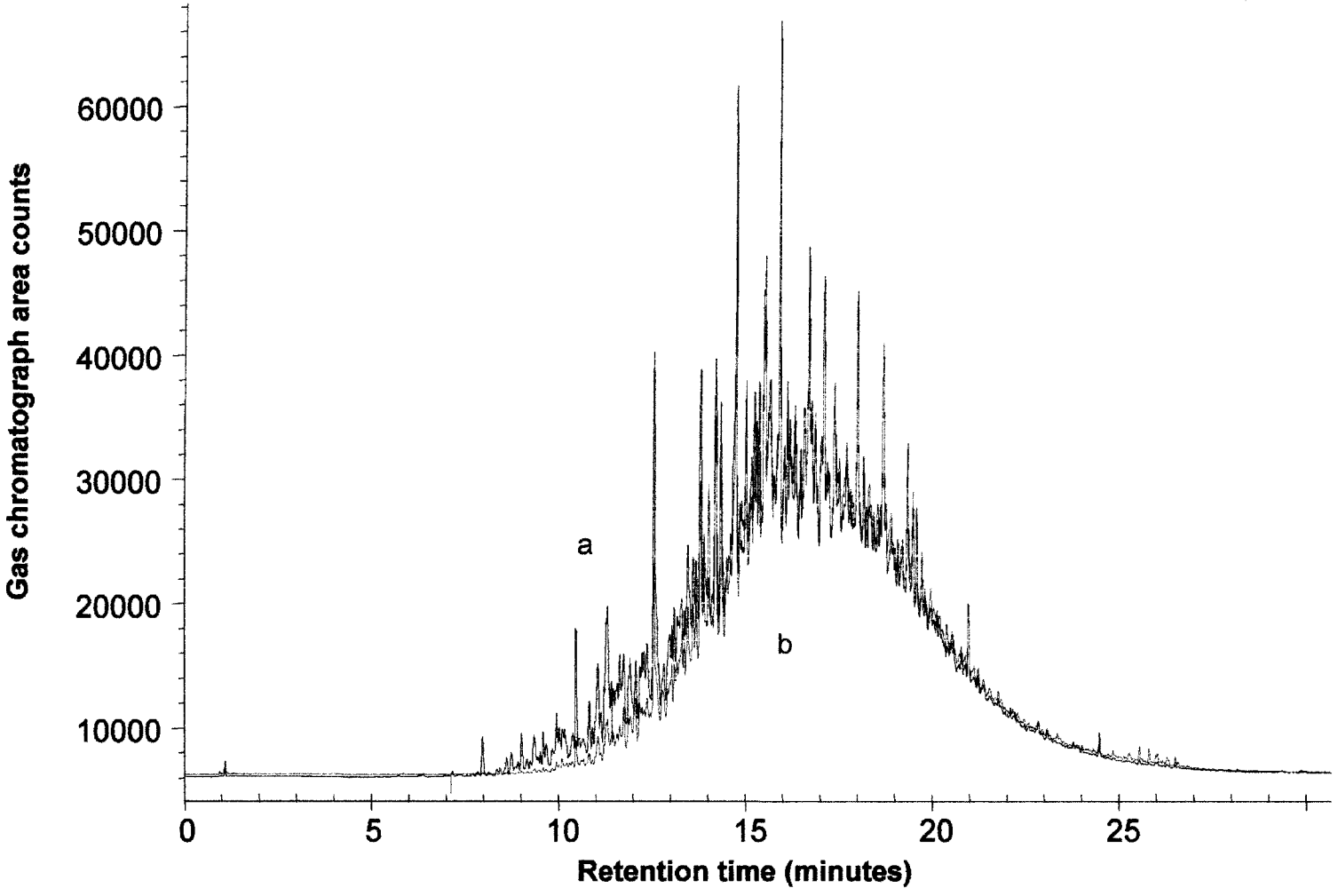

Figure 6.5. Representative chromatogram from SPME-GC-FID analysis of volatile hydrocarbons in (a) original field soil and (b) unplanted treatment soil after 16 weeks in a growth chamber.

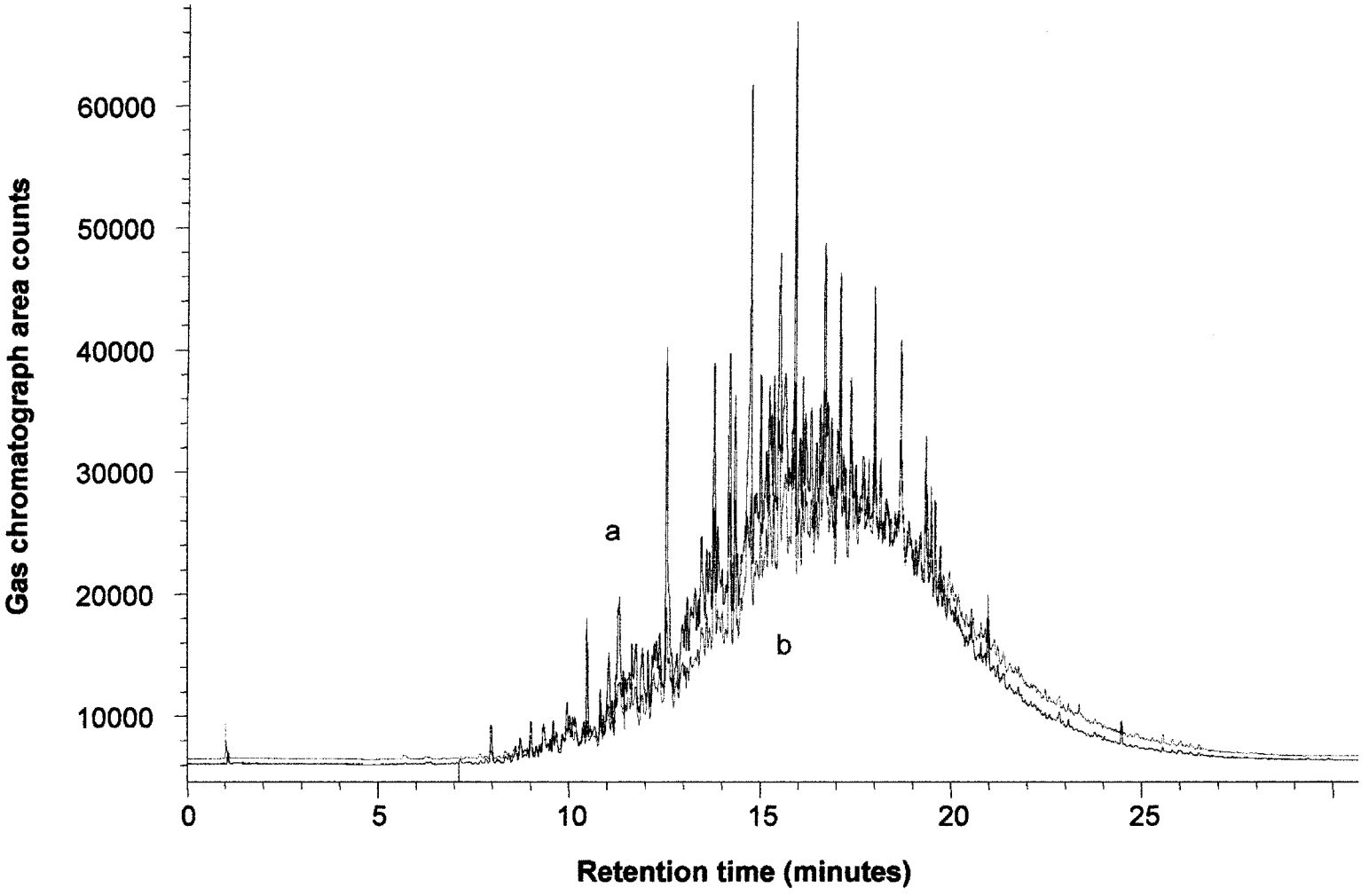

Figure 6.6. Representative chromatogram from SPME-GC-FID analysis of volatile hydrocarbons in (a) original field soil and (b) Indian breadroot treatment soil after 16 weeks in a growth chamber.

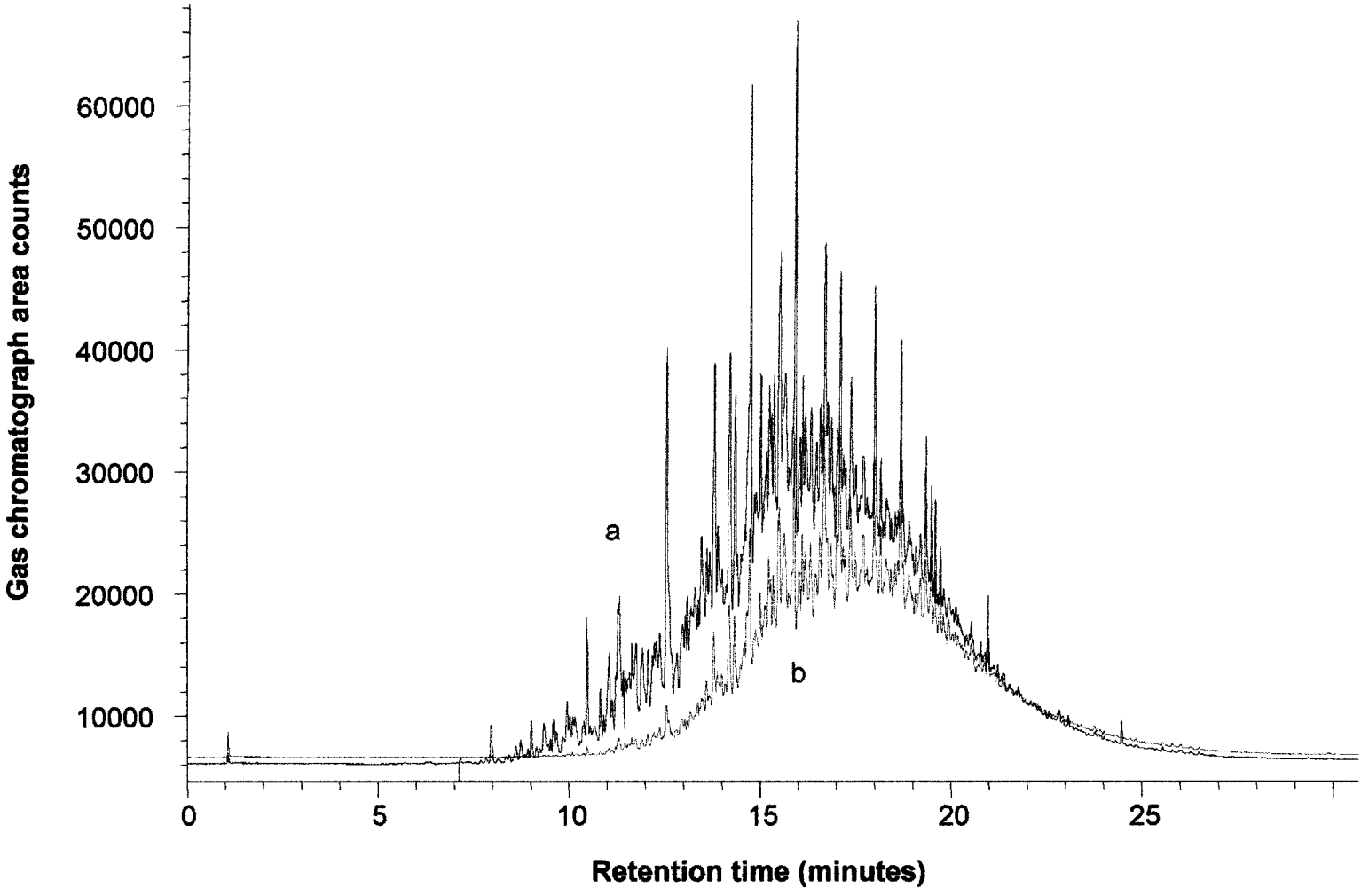

Figure 6.7. Representative chromatogram from SPME-GC-FID analysis of volatile hydrocarbons in (a) original field soil and (b) crested wheatgrass treatment soil after 16 weeks in a growth chamber.

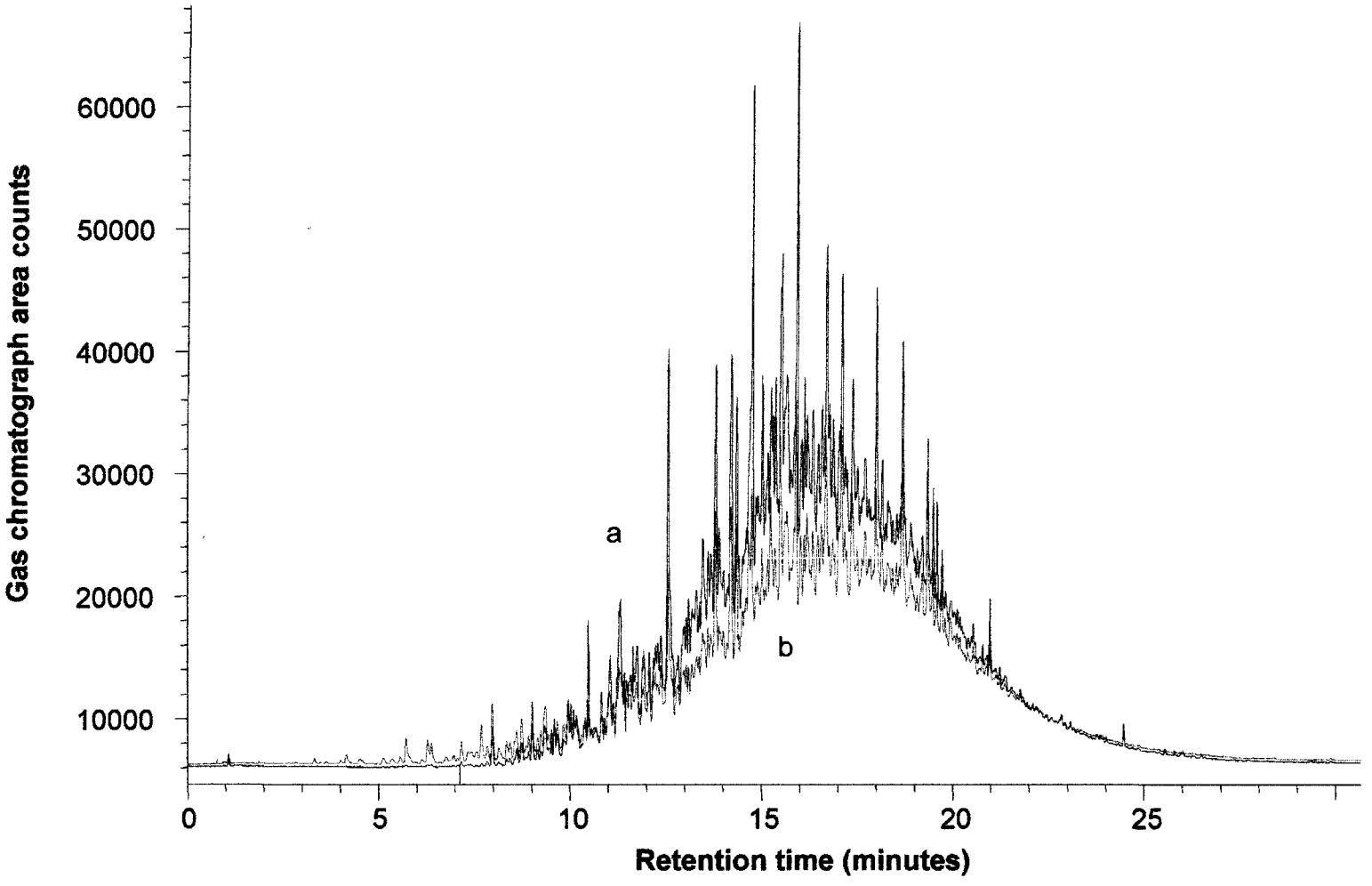

Figure 6.8. Representative chromatogram from SPME-GC-FID analysis of volatile hydrocarbons in (a) original field soil and (b) mixed Indian breadroot crested wheatgrass treatment soil after 16 weeks in a growth chamber.

wheatgrass has shallow fibrous roots and Indian breadroot deep tap roots these two species were potentially using resources from different soil depths. Thus, intraspecific competition between crested wheatgrass plants is stronger than interspecific competition with Indian breadroot plants.

Another possible reason for the greater biomass production of crested wheatgrass when grown with Indian breadroot is that the latter species facilitates the fixation of nitrogen. Active pink nodules were observed on the roots of Indian breadroot plants at harvest time. The crested wheatgrass plants in the mixed treatment may have been able to access more nitrogen than plants in the single species treatment. The relative total yield of grass plants when intercropped with legumes is typically higher than when the grass is grown in a monoculture (Martin and Snaydon, 1982; Li et al., 1999).

Biomass produced per Indian breadroot plant was the same in both single and mixed species treatments. There are two possible explanations for this observation. One is that the presence of only three plants per pot, instead of four, decreased intraspecific competition for resources in the Indian breadroot treatment. Another is that Indian breadroot plants do not compete for nutrients in the seedling stage, possibly due to the low number of lateral roots produced at this stage of growth. As legumes use nitrogen from microbial fixation (Chanway et al., 1991), competition for soil nitrogen may not be as strong as it is for grasses.

The concentration of acenaphthene was higher in the crested wheatgrass and mixed treatments compared to the original soil, and unplanted and Indian breadroot treatments. Higher concentrations of some PAHs in vegetated soil compared to

unplanted soil have been documented before (Qui et al., 1997). Qui and associates (1997) hypothesized this may be due to increased solvent extractability of PAHs due to the release of organic acids and phenols by plant roots. However, Qui and associates (1997) were using Soxhlet extraction and gas chromatography to measure changes in the PAH concentrations not SPME. Nonetheless, it is possible that acenaphthene became more volatile in response to a chemical released by crested wheatgrass. It is also possible that crested wheatgrass produced a chemical in its root exudates that had the same retention time as acenaphthene, which would have made it appear that more acenaphthene was present. Siciliano and Germida (1998b) note that there are structural similarities between some PAHs and root exudates. A third possibility is that rhizosphere bacteria degraded large hydrocarbons like 3-methylcholanthrene (Sutherland, 1992) or fluoranthene (Kelley et al., 1993) into acenaphthene, resulting in a higher concentration of the latter component. However, since the concentration of these compounds was not measured, this cannot be substantiated.

In general, small hydrocarbons have shorter retention times than larger hydrocarbons, like PAHs (Havenga and Rohwer, 1999). After the treatment period in the unplanted treatment the quantity of small hydrocarbons was slightly lower compared to the original soil, suggesting these hydrocarbons either volatilized or were degraded by microorganisms in the soil. The smaller hydrocarbon profiles of planted compared to unplanted treatments indicates that the presence of plants improved degradation of hydrocarbons in weathered contaminated field soil. In planted treatments, particularly the crested wheatgrass treatment, reduction of hydrocarbons with shorter retention times was greater than reduction of hydrocarbons with longer

retention times. When the concentrations of seven known PAHs were compared, no significant difference was noted between the treatments. This is not unexpected as higher molecular weight hydrocarbons are less likely to be degraded than small hydrocarbons (Brown et al., 1998). Large hydrocarbons are less soluble than small ones making it harder for microorganisms to degrade them (Aprill and Sims, 1990). When a selection of forage grasses and legumes were grown in weathered, contaminated sediments, which contained mostly recalcitrant hydrocarbons, no degradation occurred in 180 days (Kulakow et al., 2000). This research suggests that SPME can be useful in detecting changes in the hydrocarbon profile.

As crested wheatgrass plants caused greater degradation of weathered hydrocarbons than Indian breadroot, we would expect that higher amounts of hydrocarbons would be present in the mixed treatment than the crested wheatgrass treatment, as the soil sample from the mixed treatment had equal amounts of rhizosphere soil from each species. This is indeed the case; the hydrocarbon profile of the mixed treatment was about half way between that of Indian breadroot and crested wheatgrass treatments. Whether the decrease in quantity of hydrocarbons was due to plant degradation, storage by the plant or microbial degradation is unknown.

The reason why plants differ in their ability to stimulate degradation is unknown. It is hypothesized that plants produce chemicals that aid microorganisms in degrading soil toxicants (Walton et al., 1994). Evidence offered to support this hypothesis is that white sweet-clover (*Melilotus alba* Desr.) plants shift carbon allocation from shoots to roots when exposed to phenanthrene in soil (Walton et al., 1994). However, it is more likely that plants alter carbon allocation in reaction to the

nutrient deficiency that results from the addition a high carbon compound to the soil. Increasing root biomass improves the plant's chance of obtaining soil nutrients. Furthermore, plants may reallocate carbon to excrete chelators and organic acids to improve nutrient uptake (Lambers and Poorter, 1992).

The most likely reason why plants vary in their phytoremediation ability is that they produce different root exudates. Root exudates, allelochemicals and hydrocarbons are often structurally similar (Siciliano and Germida, 1998). A plant that normally produces allelochemicals similar to hydrocarbons may increase hydrocarbon degradation because the rhizosphere microorganisms are adapted to degrading a similar chemical. Root exudate production may change in response to stressful conditions. Plants release different root exudates in contaminated than in control soil (Haby and Crowley, 1997; Kozdroj and von Elsas, 2000). Plants also produce different quantities (Biondini et al., 1998; Warembourg and Estelrich, 2001) and kinds (Marschener, 1998; Miller et al., 2001) of exudates when grown in soils with different levels of fertility. Thus plants that do not produce exudates that aid in hydrocarbon degradation in uncontaminated soil may do so when exposed to contaminated, infertile conditions. An examination of the kinds of root exudates produced by crested wheatgrass and Indian breadroot is needed to identify any beneficial ones.

As Indian breadroot is a legume that forms root nodules, it must release root exudates like flavonoids to attract *Rhizobium* bacteria (Chanway et al., 1991). Legumes also use some of their carbon to fuel microbial fixation of nitrogen (Chanway et al., 1991). Because Indian breadroot uses carbon to attract *Rhizobium* and support nitrogen fixation, it may not be producing as many root exudates useful for hydrocarbon

degradation as species that do not fix nitrogen. Even if Indian breadroot does not increase hydrocarbon degradation by much, it may improve fertility of hydrocarbon-contaminated soil via its ability to be infected by *Rhizobium* and form nodules for symbiotic nitrogen fixation. Some fixed nitrogen is transferred from legumes to associated grasses in pastures (Ledgard and Steele, 1992). The death of legume roots is another input of nitrogen into a soil (Ledgard and Steele, 1992). Thus growing legumes and grasses together may improve overall plant productivity at a contaminated site. However, whether adding legumes to a seed mix actually improves the growth of associated plants in hydrocarbon-contaminated soil still needs to be assessed.

6.6 Conclusions

In the seedling stage of growth crested wheatgrass was responsible for more hydrocarbon degradation than Indian breadroot. Biomass production of crested wheatgrass plants was higher when grown with Indian breadroot plants, likely due to reduced competition for nutrients. Choosing a legume and a grass for phytoremediation may be beneficial for biomass production, as interspecific competition between these two plant families is likely lower than intraspecific competition. However, hydrocarbon degradation may not be as rapid when using mixed species.

7.0 GENERAL DISCUSSION

7.1 Characteristics of Hydrocarbon Tolerant Plants

The results of the growth chamber studies show that of the species tested, those most tolerant of hydrocarbon-contaminated soil were the ones with large seeds, low relative growth rates (RGRs) and the ability to fix nitrogen. Plants with these characteristics are typically adapted to stressful habitats (Grime, 1979). The hydrocarbon-contaminated soils examined in this study were considered stressful environments for plant growth because of low fertility, and sometimes high salinity. This research suggests that plants possessing stress tolerant, competitive-stress tolerant, stress tolerant-ruderal or competitive- stress tolerant-ruderal strategies (Grime, 1979) will be the most tolerant of petroleum hydrocarbon-contamination (Figure 7.1). Research testing a wider variety of species is needed to confirm or negate that general statements can be applied to many or all hydrocarbon-tolerant plants.

Plants possessing traits that confer hydrocarbon tolerance were not always common at the contaminated field plots. In the growth chamber, those species with the largest seeds (i.e. > 10 mg) were the most tolerant of hydrocarbons in soil. Species with seeds less than 0.01 mg were the least tolerant of hydrocarbons, with less than 20% of the biomass as the plants in uncontaminated soil. Large seeded species also were more common on contaminated than uncontaminated field plots. Over 60% of the plant cover on contaminated plots was from species with seeds heavier than 1 mg. However,

species with seeds heavier than 10 mg were not more common on contaminated than uncontaminated field plots. Under field conditions, many factors influence species recruitment including dispersal ability, seed production and granivory. Heavy seeds are incapable of dispersing as far as light seeds (Guo et al., 2000). Plants produce either a small number of large seeds or many small seeds (Baker, 1972; Jakobsson and Ericksson, 2000). Large seeds are also more susceptible to granivory, as they are easier for animals to find (Fenner, 1985). Thus large seeded species (>10 mg) are less abundant and may be less likely to arrive at a disturbed site, depending on their dispersal mode.

Plants with high RGRs were also more tolerant of hydrocarbon-contaminated soil in the growth chamber studies. Non-native annual species have higher RGRs than native perennial species (Christoffoleti et al., 1999; Grotkopp et al., 2002) yet non-native annuals were more common on contaminated than uncontaminated plots. This is because there are factors other than hydrocarbon contamination that affect plant survival under field conditions. Kochia (*Kochia scoparia* (L.) Schrad.) is capable of growing on nitrogen poor (McLendon and Redente, 1992), arid and saline habitats (Iverson and Wali, 1982). The soil at the contaminated plots was significantly lower in nitrogen than the uncontaminated plots and some sites were saline. Furthermore, the year 2001 was a drought year in Saskatchewan. The advantage that nitrogen acquisition ability, and drought and salt tolerance gave kochia may have made up for the disadvantage of high RGR. It should also be noted that the kochia has relatively large seeds compared to non-native annuals like lamb's-quarters (*Chenopodium album* L.) and stinkweed (*Thlaspi arvense* L.). Small seeded annuals were not common on the

hydrocarbon contaminated plots investigated, even though they thrive on disturbed soil. Kochia was dominant at the most recently disturbed plots (e.g. Cantuar) but uncommon at the oldest plots (i.e. Success 1, 2 and 3) where grasses were dominant. Other researchers note that the abundance of kochia decreases and that of grasses increases with time since disturbance possibly because grasses can reach the nutrients in deeper layers of soil than kochia (McLendon and Redente, 1992).

Legumes were among the top ranked species in survival and several were the most hydrocarbon tolerant in the growth chamber studies. There are several reasons for this superior performance. The legumes tested have relatively large seeds and slow RGR. The legumes also formed active nodules during the experiments, which would have increased available nitrogen. However, at the contaminated field plots legumes were less common than on uncontaminated plots. This may be due to lower dispersal abilities of legumes due to their large seeds, or their high initial nitrogen requirement (Fenner and Lee, 1989). The soil used in the growth chamber studies was much higher in nitrogen than the field soils. It is also possible that *Rhizobium* bacteria were scarce or less able to form active nodules in the contaminated, disturbed soil, hindering the survival of legume seedlings.

7.2 Procedure to Identify Phytoremediators

Successful phytoremediation requires first and foremost plant species that can survive in the types of soil and climatic conditions that occur at contaminated sites. A species that produces chemicals that stimulate microbial degradation of hydrocarbons but dies when exposed to freezing temperatures is clearly not suitable for use in Canada.

Therefore the first step in screening plants is to eliminate those species unlikely to survive in the region of concern. The second step is to identify species that are tolerant of hydrocarbons. Once relatively tolerant species have been selected, testing of degradation ability can begin.

How will tolerant plants be identified? This research has already determined that collecting seeds and growing them under lab conditions has limitations. Many species have high variability in seed germination. Collecting information about optimal seed treatments, normal germination patterns and ideal germination conditions is desirable before testing native plants for hydrocarbon tolerance. Another problem with growth chamber studies is that environmental conditions are not the same as in the field. Species that had poor survival in the growth chamber (e.g. western and slender wheatgrass) were among the dominant plants in the field. Under field conditions plants are exposed to drought, soils that have poor structure and low fertility and sometimes saline conditions. As it is impossible to mimic these conditions in a growth chamber, conducting field experiments are more realistic for the evaluation of hydrocarbon tolerance. The key limitation of field experiments is that they are restricted to the summer months. Finding appropriate sites for the experiments can also be problematic.

Conducting field surveys of hydrocarbon-contaminated sites that have naturally revegetated is an alternative to growth chamber/field experiments. Obviously if a plant can grow in a contaminated soil it has proven its tolerance. Sampling vegetation at hydrocarbon-contaminated sites with saline soil can result in the identification of plants tolerant of both hydrocarbons and salts. However, it must be remembered that plant colonization of contaminated sites is affected by factors other than hydrocarbon

tolerance. For example, the canonical correlation of total petroleum hydrocarbons on was lower than that of C:N ratio and available potassium suggesting that in the field other environmental variables may be more influential. Another factor affecting species composition at contaminated plots is distance to native ecosystems. Contaminated, disturbed sites will be colonized by those species whose propagules manage to reach it. The further away a disturbed site is from native vegetation, the less likely it is that native species, particularly those with vegetative reproduction, will colonize it (Kotanen, 1996; Tikka et al., 2001). One may unjustifiably conclude that native species are less tolerant of hydrocarbons than non-natives simply because the study site was isolated from native ecosystems. Thus field surveys alone may not identify the most hydrocarbon tolerant species in a region.

This research suggests there may be a third way to identify hydrocarbon tolerant species: by using ecological theory to predict which species have the highest probability of being tolerant. Three characteristics that may be indicative of hydrocarbon tolerance are: seed size, relative growth rate and mycorrhizal dependence. In this study, plants with large seeds were more tolerant of hydrocarbons than species with small seeds. Plants with seeds lighter than 0.1 mg experienced large declines in biomass when grown in contaminated compared to uncontaminated soil. This may be because small-seeded plants typically need highly fertile habitats (Westoby et al., 1990) and/or mycorrhizal associations (Allsopp and Stock, 1995) to thrive.

Determining RGR is potentially a second means to assess hydrocarbon tolerance in plants. In this study, the biomass production of species with low RGR was higher than that of species with high RGR. Species with high RGR typically perform best on

fertile soil while those with low RGR are tolerant of relatively infertile soil (Robinson and van Vuren, 1998).

Plants that are non-mycorrhizal or facultative mycotrophs may perform better on contaminated soil than obligate mycotrophs, as mycorrhizae are often less effective in contaminated, disturbed soils (Stahl et al., 1988; Jasper et al, 1989). Typically non-mycorrhizal species were more common on contaminated compared to uncontaminated field plots. Unfortunately, information on mycorrhizal status in the existing literature is sparse. It may be necessary to harvest plants from the wild to determine if they are normally mycorrhizal (Hendry and Grime, 1993). Determining mycorrhizal dependence is more difficult, requiring an experiment in which plants are grown with and without mycorrhiza and comparing their growth (Wilson et al., 1991). Before prediction can be used as a screening technique, additional testing of the hypotheses that large-seeded, low RGR and non-mycorrhizal plant species will be more tolerant of hydrocarbon-contaminated soils is needed. A wider variety of species should be tested under both growth chamber and field conditions.

If regular fertilization, tillage and addition of organic matter are feasible at a contaminated site, plants with smaller seeds (i.e. between 0.1-mg and 0.99-mg) and faster growth (i.e. between 0.6 and 0.8 mg $week^{-1}$) may be better able to survive. Many experiments have documented greatly improved plant growth with fertilizer additions (Amadi et al., 1993; Cutright, 1995; Steffenson and Alexander, 1995; Lin and Mendelssohn, 1998). Inoculation of seeds with mycorrhiza or the addition of soil that contains mycorrhizal spores and hyphae to a site may make sowing obligately mycorrhizal plants feasible. The introduction of mycorrhizal fungi along with seeds

improved plant growth on mine spoil (Lambert and Cole, 1980; Carpenter and Allen, 1988) and processed oil shale (Call and McKell, 1982). Mycorrhizae that are capable of growing in disturbed soil conditions must be selected for successful reclamation. There is evidence that some mycorrhizae are capable of degrading organic contaminants (Donnelly and Fletcher, 1994). Hydrocarbon-degrading mycorrhizal fungi would be ideal species to use for phytoremediation.

Once a suite of potential species is selected, experiments to determine which species facilitate hydrocarbon degradation should be conducted. Field trials would provide the most reliable and realistic data on degradation, as the plants would be exposed to field conditions. However, as field trials are expensive, growth chamber or greenhouse experiments may be more feasible. It is important to remember that growth conditions are more favorable in a controlled environment, so the degradation rates observed might not reflect what would really happen. Varying water, fertilizer and temperature would help to ascertain the effect of varying environmental conditions on degradation.

The results of this research suggest that hydrocarbon tolerant species are not necessarily good hydrocarbon degraders, likely because of differences in root exudate production. Thus another potential method to screen plants for degradation ability is to examine root exudates. As phenols increase hydrocarbon degradation (Hedge and Fletcher, 1987; Liste and Alexander, 1999), examining root exudates for these chemicals may be a useful screening method. The methodology developed by Liste and Alexander (1999) was simple and fairly rapid, taking only a few weeks. However, as there are likely other kinds of chemicals that also accelerate degradation, further

research into this area is required. It would be particularly useful to determine if certain plant families are more likely to produce chemicals that aid in hydrocarbon degradation than others. Plant families that are unlikely to produce appropriate root exudates could then be disregarded in the tolerance screening phase.

Once several potential phytoremediators have been identified, practical considerations regarding implementation of this biotechnology would have to be addressed. Seed availability is one of the most important considerations. If large quantities of seed are not available, it may be necessary to contract seed growers to produce seeds. Another option, if quantities of seeds are lacking or difficult to grow in large numbers, is to grow several tolerant species together rather than just one. Even if seed supply is not an issue it may be beneficial to grow mixtures of species anyway. This research suggests that growing a grass with a legume may result in greater biomass production. Greater biomass production and the presence of a nitrogen-fixing species should improve fertility of contaminated soil. Selecting plants with different rooting patterns would maximize the volume of soil explored. Planting a single shallow rooted species may not remediate the soil as well as planting a shallow and a deep rooting species, even if the deep rooting species was a less efficient hydrocarbon degrader.

In summary, the physiological traits and microbial associations of a plant species appears to influence their ability to tolerant hydrocarbons. Future research on phytoremediation ability of plants should note these features to determine if general principles can be extrapolated.

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APPENDICES

Appendix A Scientific and common names, and families of all plant species tested in growth chamber experiments (A) or sampled in field plots (B).

Scientific Name	Common Name	Plant Family	A	B
Achillea millefolium L.	Woolly yarrow	Asteraceae	X	X
Agropyron dasystachyum (Hook.) Scribn.	Northern wheatgrass	Poaceae	X	
Agropyron pectiniforme R. & S.	Crested wheatgrass	Poaceae	X	X
Agropyron smithii Rydb.	Western wheatgrass	Poaceae	X	X
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	Slender wheatgrass	Poaceae	X	X
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte	Bearded wheatgrass	Poaceae		X
Amelanchier alnifolia Nutt.	Saskatoon	Rosaceae		X
Anemone multifida Poir.	Cut-leaved anemone	Ranunculaceae		X
Antennaria parvifolia Nutt.	Small-leaved everlasting	Asteraceae		X
Arabis holboellii Hornem.	Reflexed rock-cress	Brassicaceae		X
Artemisia cana Pursh	Hoary sagebrush	Asteraceae		X
Artemisia frigida Willd.	Pasture sage	Asteraceae		X
Artemisia ludoviciana Nutt.	Prairie sage	Asteraceae		X
Aster ciliolatus Lindl.	Lindley's aster	Asteraceae	X	X
Aster ericoides L.	Many-flowered aster	Asteraceae	X	X
Aster puniceus L.	Purple-stemmed aster	Asteraceae		X
Astragalus crassicarpus Nutt.	Ground plum	Fabaceae	X	
Astragalus pectinatus Dougl. *ex* Hook.	Narrow-leaved milk-vetch	Fabaceae		X
Astragalus striatus Nutt.	Ascending purple milk-vetch	Fabaceae	X	
Avena fatua L.	Wild oat	Poaceae		X
Axyris amaranthoides L.	Russian pigweed	Chenopodiaceae		X
Bouteloua gracilis (HBK.) Lag.	Blue grama	Poaceae	X	X
Bromus ciliatus L.	Fringed brome	Poaceae	X	
Bromus inermis Leyss.	Smooth brome	Poaceae	X	X
Calamagrostis canadensis (Michx.) Beauv.	Marsh reed grass	Poaceae	X	
Campanula rotundifolia L.	Harebell	Campanulaceae		X
Carex spp.	Sedge	Cyperaceae		X
Carex spp.	Sedge	Cyperaceae		X
Carex aquatilis Wahlenb.	Water sedge	Cyperaceae		X
Chenopodium album L.	Lamb's quarters	Chenopodiaceae		X
Cirsium arvense (L.) Scop.	Canada thistle	Asteraceae		X
Cryptantha fendlerii (A. Gray) Greene	Fendler's cryptanthe	Boraginaceae		X
Descurainia sophia (L.) Webb.	Flixweed	Brassicaceae		X
Distichlis stricta (Torr.) Rydb.	Salt grass	Poaceae		X
Dracocephalum parviflorum Nutt.	American dragonhead	Lamiaceae		X
Elaeagnus commutata Bernh. *ex* Rydb.	Silverberry; wolf willow	Elaeagnaceae		X
Elymus canadensis L.	Canada wild rye	Poaceae	X	X
Elymus junceus Fisch.	Russian wild rye	Poaceae		X

Appendix A, continued

Scientific Name	Common Name	Plant Family	A	B
Epilobium angustifolium L.	Fireweed	Onagraceae	X	
Epilobium ciliatum Raf.	Northern willowherb	Onagraceae		X
Erigeron canadensis L.	Horseweed	Asteraceae		X
Erysimum asperum (Nutt.) DC.	Western wallflower	Brassicaceae		X
Euphorbia glyptosperma Engelm.	Thyme-leaved spurge	Euphorbiaceae		X
Festuca hallii (Vasey) Piper	Plains rough fescue	Poaceae	X	
Fragaria virginiana Duchesne	Wild strawberry	Rosaceae		X
Gaillardia aristata Pursh	Gaillardia	Asteraceae	X	
Galium boreale L.	Northern bedstraw	Rubiaceae	X	
Geum triflorum Pursh	Three-flowered avens	Rosaceae	X	
Glycyrrhiza lepidota (Nutt.) Pursh	Wild licorice	Fabaceae	X	X
Grindelia squarrosa (Pursh) Dunal	Gumweed	Asteraceae		X
Gutierrezia sarothrae (Pursh) Britt. & Rusby	Common broomweed	Asteraceae		X
Hedysarum alpinum L.	Hedysarum	Fabaceae	X	
Helianthus nuttallii T. & G.	Common tall sunflower	Asteraceae		X
Helianthus subrhomboideus Rydb.	Rhombic-leaved sunflower	Asteraceae	X	X
Heterotheca villosa (Pursh) Shinners	Hairy golden aster	Asteraceae	X	X
Hordeum jubatum L.	Wild barley	Poaceae		X
Iva xanthifolia Nutt.	False ragweed	Asteraceae		X
Juncus balticus Willd.	Baltic rush	Juncaceae		X
Juncus longistylis Torr.	Long-styled rush	Juncaceae		X
Kochia scoparia (L.) Schrad.	Kochia; summer cypress	Chenopodiaceae		X
Koeleria macrantha (Ledeb.) J.A. Schultes	June grass	Poaceae	X	X
Lactuca pulchella (Purdh) DC.	Common blue lettuce	Asteraceae		X
Lappula squarrosa (Retz.) Dumort.	Bluebur	Boraginaceae		X
Lathyrus venosus Muhl.	Wild peavine	Fabaceae	X	X
Lepidium densiflorum Schrad.	Common peppergrass	Brassicaceae		X
Liatris punctata Hook.	Dotted blazingstar	Asteraceae	X	X
Linum lewisii Pursh	Wild blue flax	Linaceae	X	X
Lygodesmia juncea (Pursh) D. Don	Skeletonweed	Asteraceae		X
Medicago sativa L.	Alfalfa	Fabaceae	X	X
Melilotus alba Desr.	White sweet-clover	Fabaceae		X
Melilotus officinalis (L.) Lam.	Yellow sweet-clover	Fabaceae	X	X
Mentha arvensis L.	Wild mint	Lamiaceae		X
Muhlenbergia asperifolia (Nees & Mey.) Parodi	Scratch grass	Poaceae		X
Muhlenbergia richardsonis (Trin.) Rydb.	Prairie muhly	Poaceae		X
Oenothera biennis L.	Yellow evening-primrose	Onagraceae		X
Orthocarpus luteus Nutt.	Owl's clover	Scrophulariaceae		X
Oxytropis monticola A. Gray	Late yellow locoweed	Fabaceae	X	X
Petalostemon purpureum (Vent.) Rydb.	Purple prairie-clover	Fabaceae		X
Phalaris arundinacea L.	Reed canary grass	Poaceae		X
Phleum pratense L.	Timothy	Poaceae	X	
Plantago eriopoda Torr.	Sea-side plantain	Plantaginaceae		X
Poa canbyi (Scribn.) Piper	Canby bluegrass	Poaceae		X

Appendix A, continued

Scientific Name	Common Name	Plant Family	A	B
Poa pratensis L.	Kentucky bluegrass	Poaceae	X	X
Polygonum arenastrum Jord. *ex* Bor.	Doorweed	Polygonaceae		X
Polygonum convolvulus L.	Wild buckwheat	Polygonaceae		X
Populus tremuloides Michx.	Trembling aspen	Salicaceae		X
Potentilla concinna Richards.	Early cinquefoil	Rosaceae		X
Potentilla pensylvanica L.	Prairie cinquefoil	Rosaceae	X	X
Psoralea esculenta Pursh	Indian breadroot	Fabaceae	X	X
Puccinellia nuttalliana (Schult.) A.S. Hitchc.	Nuttall's salt-meadow grass	Poaceae		X
Ratibida columnifera (Nutt.) Wooton & Standl.	Prairie coneflower	Asteraceae	X	
Rosa arkansana Porter	Prairie rose	Rosaceae		X
Rubus idaeus L.	Wild red raspberry	Rosaceae		X
Rumex occidentalis S. Wats	Western dock	Polygonaceae		X
Salicornia europaea L.	Red samphire	Chenopodiaceae		X
Salsola kali L.	Russian thistle	Chenopodiaceae		X
Scirpus paludosus A. Nels.	Prairie bulrush	Juncaceae		X
Setaria glauca (L.) Beauv.	Yellow foxtail	Poaceae		X
Setaria viridis (L.) Beauv.	Green foxtail	Poaceae		X
Smilacina stellata (L.) Desf.	False Solomon's seal	Liliaceae		X
Solidago canadensis L.	Canada goldenrod	Asteraceae		X
Solidago graminifolia (L.) Salisb.	Flat-topped goldenrod	Asteraceae		X
Solidago rigida L.	Stiff goldenrod	Asteraceae	X	X
Sonchus arvensis L.	Perennial sow-thistle	Asteraceae		X
Spartina pectinata Link	Prairie cord grass	Poaceae		X
Sphaeralcea coccinea (Pursh) Rydb.	Scarlet mallow	Malvaceae		X
Sporobolus cryptandrus (Torr.) A. Gray	Sand dropseed	Poaceae	X	
Stellaria longifolia Muhl.	Long-leaved chickweed	Caryophyllaceae		X
Stipa comata Trin. & Rupr.	Spear grass	Poaceae	X	X
Stipa curtiseta (A.S. Hitchc.) Barkworth	Porcupine grass	Poaceae	X	X
Stipa viridula Trin.	Green needle grass	Poaceae	X	X
Suaeda calceoliformis (Hook.) Moq.	Western sea-blight	Chenopodiaceae		X
Symphoricarpos occidentalis Hook.	Western snowberry	Caprifoliaceae		X
Taraxacum officinale Weber	Dandelion	Asteraceae		X
Thermopsis rhombifolia (Nutt.) Richards.	Golden bean	Fabaceae	X	X
Thlaspi arvense L.	Stinkweed	Brassicaceae		X
Tragopogon dubius Scop.	Goat's beard	Asteraceae		X
Trifolium repens L.	White clover	Fabaceae	X	
Triglochin maritima L.	Arrow-grass	Juncaginaeae		X
Vicia americana Muhl.	American vetch	Fabaceae		X

A Tested in at least one growth chamber experiment
B Found in at least one of the 28 field plots

Appendix B Alphabetical list of all plant species sampled in 28 hydrocarbon-contaminated and uncontaminated field plots and their functional characteristics.

Species	Nitrogen fixation [a]	Mycorrhizal status [b]	Origin [c]	Life form	Life span
Achillea millefolium L.	-	+	-	Herb	Perennial
Agropyron pectiniforme R. & S.	-	?	+	Herb	Perennial
Agropyron smithii Rydb.	-	+	-	Herb	Perennial
Agropyron trachycaulum (Link) Malte var. *trachycaulum* (Link) Malte	-	+	-	Herb	Perennial
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte	-	+	-	Herb	Perennial
Amelanchier alnifolia Nutt.	-	?	-	Woody	Perennial
Anemone multifida Poir.	-	?	-	Herb	Perennial
Antennaria parvifolia Nutt.	-	?	-	Herb	Perennial
Arabis holboellii Hornem.	-	-	-	Herb	Biennial
Artemisia cana Pursh	-	+	-	Woody	Perennial
Artemisia frigida Willd.	-	+	-	Herb	Perennial
Artemisia ludoviciana Nutt.	-	+	-	Herb	Perennial
Aster ciliolatus Lindl.	-	?	-	Herb	Perennial
Aster ericoides L.	-	+	-	Herb	Perennial
Aster puniceus L.	-	?	-	Herb	Perennial
Astragalus pectinatus Dougl. *ex* Hook.	+	+	-	Herb	Perennial
Avena fatua L.	-	+	+	Herb	Annual
Axyris amaranthoides L.	-	?	+	Herb	Annual
Bouteloua gracilis (HBK.) Lag.	-	+	-	Herb	Perennial
Bromus inermis Leyss.	-	+	-	Herb	Perennial
Campanula rotundifolia L.	-	+	-	Herb	Perennial
Carex spp.	-	?	-	Herb	Perennial
Carex spp.	-	?	-	Herb	Perennial
Carex aquatilis Wahlenb.	-	?	-	Herb	Perennial
Chenopodium album L.	-	-	+	Herb	Annual
Cirsium arvense (L.) Scop.	-	+	+	Herb	Perennial
Cryptantha fendlerii (A. Gray) Greene	-	?	-	Herb	Annual
Descurainia sophia (L.) Webb.	-	?	+	Herb	Annual
Distichlis stricta (Torr.) Rydb.	-	?	-	Herb	Perennial
Dracocephalum parviflorum Nutt.	-	?	-	Herb	Biennial
Elaeagnus commutata Bernh. *ex* Rydb.	+	?	-	Woody	Perennial
Elymus canadensis L.	-	?	-	Herb	Perennial
Elymus junceus Fisch.	-	?	-	Herb	Perennial
Epilobium ciliatum Raf.	-	+	-	Herb	Perennial
Erigeron canadensis L.	-	?	-	Herb	Annual
Erysimum asperum (Nutt.) DC.	-	?	-	Herb	Annual
Euphorbia glyptosperma Engelm.	-	?	-	Herb	Annual
Fragaria virginiana Duchesne	-	+	-	Herb	Perennial
Glycyrrhiza lepidota (Nutt.) Pursh	+	+	-	Herb	Perennial
Grindelia squarrosa (Pursh) Dunal	-	+	-	Herb	Biennial
Gutierrezia sarothrae (Pursh) Britt. & Rusby	-	+	-	Herb	Perennial

Appendix B, continued

Species	Nitrogen fixation [a]	Mycorrhizal status [b]	Origin [c]	Life form	Life span
Helianthus nuttallii T. & G.	-	+	-	Herb	Perennial
Heterotheca villosa (Pursh) Shinners	-	+	-	Herb	Perennial
Hordeum jubatum L.	-	?	-	Herb	Perennial
Iva xanthifolia Nutt.	-	?	-	Herb	Annual
Juncus balticus Willd.	-	-	-	Herb	Perennial
Juncus longistylis Torr.	-	?	-	Herb	Perennial
Kochia scoparia (L.) Schrad.	-	-	+	Herb	Annual
Koeleria macrantha (Ledeb.) J.A. Schultes	-	+	-	Herb	Perennial
Lactuca pulchella (Purdh) DC.	-	?	-	Herb	Perennial
Lappula squarrosa (Retz.) Dumort.	-	?	-	Herb	Annual
Lathyrus venosus Muhl.	+	+	-	Herb	Perennial
Lepidium densiflorum Schrad.	-	-	-	Herb	Annual
Liatris punctata Hook.	-	+	-	Herb	Perennial
Linum lewisii Pursh	-	+	-	Herb	Perennial
Lygodesmia juncea (Pursh) D. Don	-	+	-	Herb	Perennial
Medicago sativa L.	+	+	+	Herb	Perennial
Melilotus alba Desr.	+	+	+	Herb	Biennial
Melilotus officinalis (L.) Lam.	+	+	+	Herb	Biennial
Mentha arvensis L.	-	+	-	Herb	Perennial
Muhlenbergia asperifolia (Nees & Mey.) Parodi	-	?	-	Herb	Perennial
Muhlenbergia richardsonis (Trin.) Rydb.	-	?	-	Herb	Perennial
Oenothera biennis L.	-	+	-	Herb	Biennial
Orthocarpus luteus Nutt.	-	?	-	Herb	Annual
Oxytropis monticola A. Gray	+	+	-	Herb	Perennial
Petalostemon purpureum (Vent.) Rydb.	+	+	-	Herb	Perennial
Phalaris arundinacea L.	-	+	-	Herb	Perennial
Plantago eriopoda Torr.	-	+	-	Herb	Perennial
Poa canbyi (Scribn.) Piper	-	?	-	Herb	Perennial
Poa pratensis L.	-	+	-	Herb	Perennial
Polygonum arenastrum Jord. *ex* Bor.	-	-	-	Herb	Annual
Polygonum convolvulus L.	-	*	-	Herb	Annual
Populus tremuloides Michx.	-	+	-	Woody	Perennial
Potentilla concinna Richards.	-	+	-	Herb	Perennial
Potentilla pensylvanica L.	-	?	-	Herb	Perennial
Psoralea esculenta Pursh	+	?	-	Herb	Perennial
Puccinellia nuttalliana (Schult.) A.S. Hitchc.	-	?	-	Herb	Perennial
Rosa arkansana Porter	-	?	-	Woody	Perennial
Rubus idaeus L.	-	+	-	Woody	Perennial
Rumex occidentalis S. Wats	-	?	-	Herb	Perennial
Salicornia europaea L.	-	-	-	Herb	Annual
Salsola kali L.	-	-	+	Herb	Annual
Scirpus paludosus A. Nels.	-	?	-	Herb	Perennial
Setaria glauca (L.) Beauv.	-	?	+	Herb	Annual
Setaria viridis (L.) Beauv.	-	+	+	Herb	Annual

Appendix B, continued

Species	Nitrogen fixation [a]	Mycorrhizal status [b]	Origin [c]	Life form	Life span
Smilacina stellata (L.) Desf.	-	+	-	Herb	Perennial
Solidago canadensis L.	-	?	-	Herb	Perennial
Solidago graminifolia (L.) Salisb.	-	?	-	Herb	Perennial
Solidago rigida L.	-	+	-	Herb	Perennial
Sonchus arvensis L.	-	+	+	Herb	Perennial
Spartina pectinata Link	-	?	-	Herb	Perennial
Sphaeralcea coccinea (Pursh) Rydb.	-	+	-	Herb	Perennial
Stellaria longifolia Muhl.	-	?	-	Herb	Perennial
Stipa comata Trin. & Rupr.	-	+	-	Herb	Perennial
Stipa curtiseta (A.S. Hitchc.) Barkworth	-	?	-	Herb	Perennial
Stipa viridula Trin.	-	?	-	Herb	Perennial
Suaeda calceoliformis (Hook.) Moq.	-	?	-	Herb	Annual
Symphoricarpos occidentalis Hook.	-	?	-	Woody	Perennial
Taraxacum officinale Weber	-	+	+	Herb	Perennial
Thermopsis rhombifolia (Nutt.) Richards.	+	+	-	Herb	Perennial
Thlaspi arvense L.	-	-	+	Herb	Annual
Tragopogon dubius Scop.	-	+	+	Herb	Biennial
Triglochin maritima L.	-	?	-	Herb	Perennial
Vicia americana Muhl.	+	+	-	Herb	Perennial

[a] Nitrogen Fixation
+ forms an association with nitrogen-fixing bacteria
- does not form an association with nitrogen-fixing bacteria

[b] Mycorrhizal Status
+ typically forms an association with mycorrhizal fungi
- typically does not form an association with mycorrhizal fungi
? ability to form an association with mycorrhiza unknown

[c] Origin
+ did not grow naturally in North America prior to European colonization
- did grow naturally in North America prior to European colonization

Appendix B, continued

Species	Reproduction mode [d]	Pollination mode	Dispersal mode [e]	Seed size [f]
Achillea millefolium L.	Vegetative	Insect	Wind	4
Agropyron pectiniforme R. & S.	By seed only	Wind	Mammal	2
Agropyron smithii Rydb.	Vegetative	Wind	Mammal	2
Agropyron trachycaulum (Link) Malte var. *trachycaulum* (Link) Malte	Vegetative	Wind	Mammal	2
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte	By seed only	Wind	Mammal	2
Amelanchier alnifolia Nutt.	By seed only	Insect	Bird	2
Anemone multifida Poir.	Seed only	Insect	Wind	2
Antennaria parvifolia Nutt.	Vegetative	Insect	Wind	3
Arabis holboellii Hornem.	By seed only	Insect	Unassisted	3
Artemisia cana Pursh	By seed only	Wind	Unassisted	3
Artemisia frigida Willd.	By seed only	Wind	Unassisted	4
Artemisia ludoviciana Nutt.	Vegetative	Wind	Unassisted	4
Aster ciliolatus Lindl.	Vegetative	Insect	Wind	3
Aster ericoides L.	By seed only	Insect	Wind	3
Aster puniceus L.	Vegetative	Insect	Wind	3
Astragalus pectinatus Dougl. *ex* Hook.	By seed only	Insect	Unassisted	2
Avena fatua L.	By seed only	Wind	Unassisted	1
Axyris amaranthoides L.	By seed only	Wind	Wind	2
Bouteloua gracilis (HBK.) Lag.	By seed only	Wind	Mammal	3
Bromus inermis Leyss.	Vegetative	Wind	Wind	2
Campanula rotundifolia L.	By seed only	Insect	Unassisted	4
Carex spp.	By seed only	Wind	Unassisted	3
Carex spp.	By seed only	Wind	Unassisted	3
Carex aquatilis Wahlenb.	Vegetative	Wind	Unassisted	3
Chenopodium album L.	By seed only	Self	Mammal	3
Cirsium arvense (L.) Scop.	By seed only	Insect	Wind	2
Cryptantha fendlerii (A. Gray) Greene	By seed only	Insect	Mammal	2
Descurainia sophia (L.) Webb.	By seed only	Insect	Unassisted	3
Distichlis stricta (Torr.) Rydb.	Vegetative	Wind	Mammal	3
Dracocephalum parviflorum Nutt.	By seed only	Insect	Unassisted	3
Elaeagnus commutata Bernh. *ex* Rydb.	Vegetative	Insect	Bird	1
Elymus canadensis L.	Vegetative	Wind	Mammal	2
Elymus junceus Fisch.	By seed only	Wind	Mammal	2
Epilobium ciliatum Raf.	By seed only	Self	Wind	4
Erigeron canadensis L.	By seed only	Insect	Wind	4
Erysimum asperum (Nutt.) DC.	By seed only	Insect	Unassisted	3
Euphorbia glyptosperma Engelm.	By seed only	Self	Unassisted	3
Fragaria virginiana Duchesne	Vegetative	Self	Bird	3
Glycyrrhiza lepidota (Nutt.) Pursh	By seed only	Insect	Mammal	3
Grindelia squarrosa (Pursh) Dunal	By seed only	Insect	Wind	3
Gutierrezia sarothrae (Pursh) Britt. & Rusby	By seed only	Insect	Wind	3
Helianthus nuttallii T. & G.	By seed only	Insect	Wind	2

Appendix B, continued

Species	Reproduction mode [d]	Pollination mode	Dispersal mode [e]	Seed size [f]
Heterotheca villosa (Pursh) Shinners	By seed only	Insect	Wind	3
Hordeum jubatum L.	By seed only	Self	Wind	2
Iva xanthifolia Nutt.	By seed only	Wind	Wind	3
Juncus balticus Willd.	Vegetative	Wind	Wind	4
Juncus longistylis Torr.	Vegetative	Wind	Wind	4
Kochia scoparia (L.) Schrad.	By seed only	Wind	Wind	2
Koeleria macrantha (Ledeb.) J.A. Schultes	By seed only	Wind	Mammal	3
Lactuca pulchella (Purdh) DC.	By seed only	Insect	Wind	3
Lappula squarrosa (Retz.) Dumort.	By seed only	Insect	Mammal	2
Lathyrus venosus Muhl.	Vegetative	Insect	Unassisted	1
Lepidium densiflorum Schrad.	By seed only	Insect	Unassisted	3
Liatris punctata Hook.	By seed only	Insect	Wind	2
Linum lewisii Pursh	By seed only	Insect	Unassisted	3
Lygodesmia juncea (Pursh) D. Don	By seed only	Insect	Wind	3
Medicago sativa L.	By seed only	Insect	Mammal	2
Melilotus alba Desr.	By seed only	Self	Mammal	2
Melilotus officinalis (L.) Lam.	By seed only	Insect	Mammal	2
Mentha arvensis L.	Vegetative	Insect	Unassisted	3
Muhlenbergia asperifolia (Nees & Mey.) Parodi	Vegetative	Wind	Wind	3
Muhlenbergia richardsonis (Trin.) Rydb.	Vegetative	Wind	Wind	3
Oenothera biennis L.	By seed only	Insect	Unassisted	3
Orthocarpus luteus Nutt.	By seed only	Insect	Wind	3
Oxytropis monticola A. Gray	By seed only	Insect	Unassisted	3
Petalostemon purpureum (Vent.) Rydb.	By seed only	Insect	Unassisted	2
Phalaris arundinacea L.	Vegetative	Wind	Wind	3
Plantago eriopoda Torr.	Vegetative	Insect	Mammal	3
Poa canbyi (Scribn.) Piper	By seed only	Wind	Unassisted	3
Poa pratensis L.	Vegetative	Wind	Unassisted	3
Polygonum arenastrum Jord. *ex* Bor.	By seed only	Self	Bird	2
Polygonum convolvulus L.	By seed only	Self	Bird	2
Populus tremuloides Michx.	Vegetative	Wind	Wind	4
Potentilla concinna Richards.	By seed only	Insect	Unassisted	3
Potentilla pensylvanica L.	By seed only	Insect	Unassisted	4
Psoralea esculenta Pursh	By seed only	Insect	Unassisted	1
Puccinellia nuttalliana (Schult.) A.S. Hitchc.	By seed only	Wind	Unassisted	3
Rosa arkansana Porter	By seed only	Insect	Mammal	2
Rubus idaeus L.	By seed only	Insect	Mammal	3
Rumex occidentalis S. Wats	By seed only	Insect	Wind	3
Salicornia europaea L.	By seed only	Insect	Unassisted	3
Salsola kali L.	By seed only	Insect	Wind	2
Scirpus paludosus A. Nels.	Vegetative	Wind	Unassisted	2
Setaria glauca (L.) Beauv.	By seed only	Self	Mammal	2
Setaria viridis (L.) Beauv.	By seed only	Self	Mammal	2

Appendix B, continued

Species	Re production mode [d]	Pollination mode	Dispersal mode [e]	Seed size [f]
Smilacina stellata (L.) Desf.	Vegetative	Insect	Mammal	2
Solidago canadensis L.	Vegetative	Self	Wind	3
Solidago graminifolia (L.) Salisb.	Vegetative	Insect	Wind	4
Solidago rigida L.	By seed only	Insect	Wind	3
Sonchus arvensis L.	By seed only	Insect	Wind	3
Spartina pectinata Link	Vegetative	Wind	Unassisted	3
Sphaeralcea coccinea (Pursh) Rydb.	Vegetative	Insect	Unassisted	3
Stellaria longifolia Muhl.	By seed only	Insect	Unassisted	3
Stipa comata Trin. & Rupr.	By seed only	Wind	Mammal	2
Stipa curtiseta (A.S. Hitchc.) Barkworth	By seed only	Wind	Mammal	2
Stipa viridula Trin.	By seed only	Wind	Mammal	2
Suaeda calceoliformis (Hook.) Moq.	By seed only	Insect	Unassisted	3
Symphoricarpos occidentalis Hook.	Vegetative	Insect	Bird	2
Taraxacum officinale Weber	By seed only	Self	Wind	3
Thermopsis rhombifolia (Nutt.) Richards.	Vegetative	Insect	Unassisted	1
Thlaspi arvense L.	By seed only	Self	Bird	3
Tragopogon dubius Scop.	By seed only	Self	Wind	2
Triglochin maritima L.	By seed only	Insect	Mammal	3
Vicia americana Muhl.	By seed only	Insect	Unassisted	1

[c] Reproduction Mode
Vegetative = Plants that reproduce both via seed production and by production of rhizomes or stolons
By seed only = Plants that reproduce via seed production only

[d] Seed Dispersal
Bird = Plants whose seeds are dispersed mainly via consumption by birds
Mammal = Plants whose seeds are dispersed via consumption, collection and subsequent storage or adhering to the fur of mammals
Unassisted = Plants whose seeds have no obvious means of improving their dispersal
Wind = Plants whose seeds are very small and light or that possess structures (i.e. wings, hairs, etc.) that improve dispersal by wind

[e] Seed Size
1 - > 10 mg
2 - 9.99 mg to 1 mg
3 - 0.99 mg to 0.1 mg
4 - 0.099 mg to 0.01 mg

Appendix C1 Alphabetical list of all plant species sampled in 14 hydrocarbon-contaminated plots. All values are relative vegetation cover.

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Achillea millefolium L.	0.13							
Agropyron pectiniforme R. & S. *	2.005	0.005			35.5			
Agropyron smithii Rydb.					0.5		0.25	
Agropyron trachycaulum (Link) Malte var. *trachycaulum*		8.25				0.75		5
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte	0.005							
Arabis holboellii Hornem.	0.38							
Artemisia cana Pursh					0.005			
Artemisia frigida Willd.	0.265			0.125				
Artemisia ludoviciana Nutt.	0.005	0.01						
Aster ciliolatus Lindl.								
Aster ericoides L.	1.54	0.29		1.14		0.005	0.145	0.005
Avena fatua L. *			0.005					
Axyris amaranthoides L. *			0.005					
Bouteloua gracilis (HBK.) Lag.				0.125				
Bromus inermis Leyss. *	5.375	0.755				0.38		
Carex spp.				0.055				
Carex spp.						0.005		
Chenopodium album L. *					0.005		0.26	
Cirsium arvense (L.) Scop. *	0.16					2.38		
Cryptantha fendlerii (A. Gray) Greene								
Descurainia sophia (L.) Webb. *								
Distichlis stricta (Torr.) Rydb.		0.005						
Dracocephalum parviflorum Nutt.						0.005		
Elaeagnus commutata Bernh. *ex* Rydb.				0.875				
Elymus canadensis L.		0.055				0.25		
Elymus junceus Fisch.				3.625				
Erigeron canadensis L.		0.155		0.025				

Appendix C1
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Erysimum asperum (Nutt.) DC.	0.005							
Euphorbia glyptosperma Engelm.					0.005			
Glycyrrhiza lepidota (Nutt.) Pursh					0.5			
Grindelia squarrosa (Pursh) Dunal	1.135	0.005		0.005	0.005	6.63	0.405	
Gutierrezia sarothrae (Pursh) Britt. & Rusby					0.005			
Helianthus nuttallii T. & G.					0.005			
Heterotheca villosa (Pursh) Shinners	1.135				0.135			
Hordeum jubatum L.	1.39	3	0.005	0.005		6.38	35.25	3.75
Juncus longistylis Torr.		0.1						
Kochia scoparia (L.) Schrad. *		0.005	38.13	34.375	0.005	0.005	11.375	
Koeleria macrantha (Ledeb.) J.A. Schultes f.	0.875							
Lepidium densiflorum Schrad.								
Lygodesmia juncea (Pursh) D. Don					0.005			
Medicago sativa L. *	0.015	0.005					0.145	
Melilotus alba Desr. *	0.015							
Melilotus officinalis (L.) Lam. *	0.01		0.01		0.51	0.015	0.005	
Petalostemon purpureum (Vent.) Rydb.		0.005						
Plantago eriopoda Torr.								1.88
Poa canbyi (Scribn.) Piper					0.125			
Poa pratensis L.	16.125	2.875		14.37		7		
Polygonum arenastrum Jord. *ex* Bor.							0.015	
Polygonum convolvulus L.					0.005			
Puccinellia nuttalliana (Schult.) A.S. Hitchc.		0.25						5
Rosa arkansana Porter								
Rumex stenophyllus Ledeb.	0.005							
Salicornia europaea L.								9.76
Salsola kali L. *			0.255					
Scirpus paludosus A. Nels.								7.625
Setaria glauca (L.) Beauv. *				0.125				

Appendix C1
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Setaria viridis (L.) Beauv. *					0.005			
Solidago canadensis L.	1.01	0.255				0.1		
Solidago graminifolia (L.) Salisb.					0.755			
Sonchus arvensis L. *	0.15	7.25				0.505		
Sphaeralcea coccinea (Pursh) Rydb.					0.005			
Suaeda calceoliformis (Hook.) Moq.							0.005	1.89
Symphoricarpos occidentalis Hook.					0.005	0.005		
Taraxacum officinale Weber *	3.635	0.001				0.28	0.01	
Thermopsis rhombifolia (Nutt.) Richards.					0.005			
Thlaspi arvense L.*					0.135			
Tragopogon dubius Scop. *	0.005			0.125	0.125			
Triglochin maritima L.						0.005	0.005	

Appendix C1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Achillea millefolium L.			0.005			
Agropyron pectiniforme R. & S. *	0.875			1		0.38
Agropyron smithii Rydb.		0.1		16.5	28.75	22.5
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	1.01		0.125	10.625		34.25
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte				0.1		
Arabis holboellii Hornem.						
Artemisia cana Pursh						
Artemisia frigida Willd.				0.005	2.26	
Artemisia ludoviciana Nutt.						
Aster ericoides L.	0.005	0.005	0.13			
Avena fatua L. *						
Axyris amaranthoides L. *						
Bouteloua gracilis (HBK.) Lag.						
Bromus inermis Leyss. *	0.005			2.755	21	
Carex spp.						
Carex spp.						
Chenopodium album L. *		0.005		0.005		0.145
Cirsium arvense (L.) Scop. *				0.03	0.05	0.02
Cryptantha fendlerii (A. Gray) Greene				1.63		0.52
Descurainia sophia (L.) Webb. *				0.005	0.01	
Distichlis stricta (Torr.) Rydb.	32.125	16.75	25.52			
Dracocephalum parviflorum Nutt.						
Elaeagnus commutata Bernh. *ex* Rydb.				0.005		
Elymus canadensis L.						
Elymus junceus Fisch.						
Erigeron canadensis L.					0.01	
Erysimum asperum (Nutt.) DC.						

Appendix C1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Euphorbia glyptosperma Engelm.						
Glycyrrhiza lepidota (Nutt.) Pursh						
Grindelia squarrosa (Pursh) Dunal	0.01	0.005	1.02			
Gutierrezia sarothrae (Pursh) Britt. & Rusby						
Helianthus nuttallii T. & G.						
Heterotheca villosa (Pursh) Shinners					0.175	
Hordeum jubatum L.		26.26	0.125			
Juncus longistylis Torr.						
Kochia scoparia (L.) Schrad. *	0.02	1.26	0.255	3.015		0.915
Koeleria macrantha (Ledeb.) J.A. Schultes f.						
Lepidium densiflorum Schrad.	0.005					
Lygodesmia juncea (Pursh) D. Don						
Medicago sativa L. *				0.13	0.015	
Melilotus alba Desr. *						
Melilotus officinalis (L.) Lam. *						
Petalostemon purpureum (Vent.) Rydb.						
Plantago eriopoda Torr.						
Poa canbyi (Scribn.) Piper	40.5	4.88	0.13			
Poa pratensis L.				2.125	2.765	
Polygonum arenastrum Jord. *ex* Bor.						
Polygonum convolvulus L.						
Puccinellia nuttalliana (Schult.) A.S. Hitchc.			0.755			
Rosa arkansana Porter				0.005		
Rumex stenophyllus Ledeb.						
Salicornia europaea L.						
Salsola kali L. *						
Scirpus paludosus A. Nels.						
Setaria glauca (L.) Beauv. *						
Setaria viridis (L.) Beauv. *						

Appendix C1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Solidago canadensis L.						
Solidago graminifolia (L.) Salisb.						
Sonchus arvensis L. *						
Sphaeralcea coccinea (Pursh) Rydb.						
Suaeda calceoliformis (Hook.) Moq.						
Symphoricarpos occidentalis Hook.				0.005		
Taraxacum officinale Weber *						
Thermopsis rhombifolia (Nutt.) Richards.						
Thlaspi arvense L.*						
Tragopogon dubius Scop. *	0.015	0.005				
Triglochin maritima L.						

*exotic

Appendix C2 Alphabetical list of all plant species sampled in 14 uncontaminated plots. All values are relative vegetation cover.

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Achillea millefolium L.		0.165		0.66				
Agropyron pectiniforme R. & S. *	0.375		0.25		15.125			
Agropyron smithii Rydb.	2.75			7				
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	3	17.625	0.005		4.625	5.375		4.13
Agropyron trachycaulum (Link) Malte var. *unilaterale (Cassidy) Malte*				1.505		0.055		
Amelanchier alnifolia Nutt.						0.125		
Anemone multifida Poir.	0.005	0.01						
Antennaria parvifolia Nutt.		0.055		0.125				
Arabis holboellii Hornem.								
Artemisia cana Pursh					0.005			
Artemisia frigida Willd.	0.67			0.265	0.005			
Artemisia ludoviciana Nutt.	0.005	0.14		0.135				
Aster ericoides L.	1.895	0.81		0.405	0.01		0.055	
Aster puniceus L.		0.035						2.65
Astragalus pectinatus Dougl. *ex* Hook.				0.005				
Avena fatua L. *					0.005			
Bouteloua gracilis (HBK.) Lag.	8.25			16.125				
Bromus inermis Leyss. *	2.88		8.75		6.625	0.055	15.875	
Campanula rotundifolia L.		0.005						
Carex aquatilis Wahlenb.								38.375
Chenopodium album L. *							4.125	
Cirsium arvense (L.) Scop. *		0.265				0.55		1.145
Cryptantha fendlerii (A. Gray) Greene								
Descurainia sophia (L.) Webb. *					0.005		0.005	
Distichlis stricta (Torr.) Rydb.								
Elaeagnus commutata Bernh. *ex* Rydb.	0.28	1.135		6.02				
Elymus canadensis L.			0.01					

Appendix C2
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Erigeron canadensis L.								
Fragaria virginiana Duchesne		0.005				1.26		
Glycyrrhiza lepidota (Nutt.) Pursh								
Grindelia squarrosa (Pursh) Dunal					0.27	0.15	0.395	
Gutierrezia sarothrae (Pursh) Britt. & Rusby	1.01							
Helianthus nuttallii T. & G.								0.01
Heterotheca villosa (Pursh) Shinners	0.52				0.375			
Hordeum jubatum L.			0.01		3.885	0.1	11.875	0.85
Iva xanthifolia Nutt.								
Juncus balticus Willd.								2.75
Kochia scoparia (L.) Schrad. *			0.005		0.005		3.375	
Koeleria macrantha (Ledeb.) J.A. Schultes f.	3.5							
Lactuca pulchella (Purdh) DC.					0.01			
Lathyrus venosus Muhl.						0.01		
Liatris punctata Hook.	0.005							
Linum lewisii Pursh	0.005	0.025						
Medicago sativa L. *					0.125		18.125	
Melilotus alba Desr. *	0.125							
Melilotus officinalis (L.) Lam. *					1.13	0.16		
Mentha arvensis L.								0.005
Muhlenbergia asperifolia (Nees & Mey.) Parodi		4						
Muhlenbergia richardsonis (Trin.) Rydb.		12						
Orthocarpus luteus Nutt.		0.005		0.005				
Oxytropis monticola A. Gray	0.005							
Petalostemon purpureum (Vent.) Rydb.	0.01	0.01		0.01				
Phalaris arundinacea L.								8.5
Plantago eriopoda Torr.							0.25	

Appendix C2
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Poa canbyi (Scribn.) Piper								
Poa pratensis L.	11.75	10.875	42.875	22.25	15.39	30.5		
Populus tremuloides Michx.				0.005		0.385		
Potentilla concinna Richards.								
Potentilla pensylvanica L.	0.01	0.005						
Psoralea esculenta Pursh	0.015			0.005				
Puccinellia nuttalliana (Schult.) A.S. Hitchc.								
Rosa arkansana Porter	0.285	0.155		0.03				
Rubus idaeus L.						1.005		
Rumex occidentalis S. Wats								
Salicornia europaea L.							0.14	
Setaria viridis (L.) Beauv. *					0.135			
Smilacina stellata (L.) Desf.		0.005						
Solidago canadensis L.	0.135	1.39						
Solidago graminifolia (L.) Salisb.								0.005
Solidago rigida L.	0.005							
Sonchus arvensis L. *		0.02					0.01	1.505
Spartina pectinata Link		0.005						11.75
Stellaria longifolia Muhl.								
Stipa comata Trin. & Rupr.	1			2.875				
Stipa curtiseta (A.S. Hitchc.) Barkworth	11.375							
Stipa viridula Trin.	2							
Suaeda calceoliformis (Hook.) Moq.							0.125	
Symphoricarpos occidentalis Hook.		7.255		6.5		7.625		
Taraxacum officinale Weber *		0.005		0.005		0.52	0.005	
Thermopsis rhombifolia (Nutt.) Richards.				0.015				
Tragopogon dubius Scop. *	0.005					0.005		
Vicia americana Muhl.		0.005						

Appendix C2
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Achillea millefolium L.			0.01	0.01		0.015
Agropyron pectiniforme R. & S. *		2.75				
Agropyron smithii Rydb.		0.755		29.125	26	21
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	1.874	37.875	3.005			
Agropyron trachycaulum (Link) Malte var. *unilaterale (Cassidy) Malte*						
Amelanchier alnifolia Nutt.						
Anemone multifida Poir.						
Antennaria parvifolia Nutt.				1.5		0.505
Arabis holboellii Hornem.				0.005		
Artemisia cana Pursh						
Artemisia frigida Willd.		0.125			1.28	
Artemisia ludoviciana Nutt.						
Aster ericoides L.		0.01	3.015			
Aster puniceus L.						
Astragalus pectinatus Dougl. *ex* Hook.						
Avena fatua L. *						
Bouteloua gracilis (HBK.) Lag.						
Bromus inermis Leyss. *		0.875		4.375	15	11.375
Campanula rotundifolia L.						
Carex aquatilis Wahlenb.						
Chenopodium album L. *	0.125					
Cirsium arvense (L.) Scop. *				0.145		0.145
Cryptantha fendlerii (A. Gray) Greene				0.01		
Descurainia sophia (L.) Webb. *			0.01	0.005	0.005	
Distichlis stricta (Torr.) Rydb.	26		10.875			
Elaeagnus commutata Bernh. *ex* Rydb.						0.01
Elymus canadensis L.						

Appendix C2
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Erigeron canadensis L.					0.005	
Fragaria virginiana Duchesne						
Glycyrrhiza lepidota (Nutt.) Pursh		0.01				
Grindelia squarrosa (Pursh) Dunal	0.125		0.145			
Gutierrezia sarothrae (Pursh) Britt. & Rusby						
Helianthus nuttallii T. & G.						
Heterotheca villosa (Pursh) Shinners	0.005	7.25			0.415	0.005
Hordeum jubatum L.	13.625		9.885			
Iva xanthifolia Nutt.	3.75					
Juncus balticus Willd.		0.26				
Kochia scoparia (L.) Schrad. *						
Koeleria macrantha (Ledeb.) J.A. Schultes f.						
Lactuca pulchella (Purdh) DC.						
Lathyrus venosus Muhl.						
Liatris punctata Hook.						
Linum lewisii Pursh						
Medicago sativa L. *				0.005	0.265	0.64
Melilotus alba Desr. *						
Melilotus officinalis (L.) Lam. *			0.005			
Mentha arvensis L.						
Muhlenbergia asperifolia (Nees & Mey.) Parodi						
Muhlenbergia richardsonis (Trin.) Rydb.						
Orthocarpus luteus Nutt.						
Oxytropis monticola A. Gray						
Petalostemon purpureum (Vent.) Rydb.						
Phalaris arundinacea L.						
Plantago eriopoda Torr.						

Appendix C2
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Poa canbyi (Scribn.) Piper	5	3.755	36.625			
Poa pratensis L.				12.25	11.635	19
Populus tremuloides Michx.						
Potentilla concinna Richards.				0.005		
Potentilla pensylvanica L.				0.01		0.03
Psoralea esculenta Pursh						
Puccinellia nuttalliana (Schult.) A.S. Hitchc.	19		0.5			
Rosa arkansana Porter				1.875		0.255
Rubus idaeus L.						
Rumex occidentalis S. Wats			0.05			
Salicornia europaea L.						
Setaria viridis (L.) Beauv. *						
Smilacina stellata (L.) Desf.						
Solidago canadensis L.						0.055
Solidago graminifolia (L.) Salisb.				0.005		
Solidago rigida L.						
Sonchus arvensis L. *	0.125		0.01			
Spartina pectinata Link						
Stellaria longifolia Muhl.					0.005	0.005
Stipa comata Trin. & Rupr.						
Stipa curtiseta (A.S. Hitchc.) Barkworth						
Stipa viridula Trin.						
Suaeda calceoliformis (Hook.) Moq.	0.255					
Symphoricarpos occidentalis Hook.				9.65		2
Taraxacum officinale Weber *						
Thermopsis rhombifolia (Nutt.) Richards.						
Tragopogon dubius Scop. *			0.005			
Vicia americana Muhl.				0.005		

* exotic

Appendix D1 Alphabetical list of all plant species sampled in 14 hydrocarbon-contaminated plots. All values are frequency (% quadrats with at least one individual)

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Achillea millefolium L.	10							
Agropyron pectiniforme R. & S. *	15	5			100			
Agropyron smithii Rydb.					20		10	
Agropyron trachycaulum (Link) Malte var. *trachycaulum*		65				30		5
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte	5							
Arabis holboellii Hornem.	20							
Artemisia cana Pursh					5			
Artemisia frigida Willd.	25			5				
Artemisia ludoviciana Nutt.	5	10						
Aster ericoides L.	75	50		40		5	25	5
Avena fatua L. *			25					
Axyris amaranthoides L. *			5					
Bouteloua gracilis (HBK.) Lag.				5				
Bromus inermis Leyss. *	30	10				20		
Carex spp.				10				
Carex spp.						5		
Chenopodium album L. *					5		20	
Cirsium arvense (L.) Scop. *	40					50		
Cryptantha fendlerii (A. Gray) Greene								
Descurainia sophia (L.) Webb. *								
Distichlis stricta (Torr.) Rydb.		5						
Dracocephalum parviflorum Nutt.						5		
Elaeagnus commutata Bernh. *ex* Rydb.				10				
Elymus canadensis L.		10				10		
Elymus junceus Fisch.				25				
Erigeron canadensis L.		35		25				
Erysimum asperum (Nutt.) DC.	5							

Appendix D1
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Euphorbia glyptosperma Engelm.					5			
Glycyrrhiza lepidota (Nutt.) Pursh					20			
Grindelia squarrosa (Pursh) Dunal	30	5		5	5	95	45	
Gutierrezia sarothrae (Pursh) Britt. & Rusby					5			
Helianthus nuttallii T. & G.					5			
Heterotheca villosa (Pursh) Shinners	30				15			
Hordeum jubatum L.	45	45	5	5		65	100	10
Juncus longistylis Torr.		10						
Kochia scoparia (L.) Schrad. *		5	100	80	5	5	55	
Koeleria macrantha (Ledeb.) J.A. Schultes f.	10							
Lepidium densiflorum Schrad.								
Lygodesmia juncea (Pursh) D. Don					5			
Medicago sativa L. *	15	5					25	
Melilotus alba Desr. *	15							
Melilotus officinalis (L.) Lam. *	10		5		30	15	5	
Oenothera biennis L.								
Petalostemon purpureum (Vent.) Rydb.		5						
Plantago eriopoda Torr.								30
Poa canbyi (Scribn.) Piper					5			
Poa pratensis L.	70	40		50		40		
Polygonum arenastrum Jord. *ex* Bor.							15	
Polygonum convolvulus L.					5			
Puccinellia nuttalliana (Schult.) A.S. Hitchc.		10						25
Rosa arkansana Porter								
Rumex stenophyllus Ledeb.	5							
Salicornia europaea L.								65
Salsola kali L. *			15					
Scirpus paludosus A. Nels.								20
Setaria glauca (L.) Beauv.				5				

Appendix D1
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Setaria viridis (L.) Beauv. *					5			
Solidago canadensis L.	25	15				10		
Solidago graminifolia (L.) Salisb.					10			
Sonchus arvensis L. *	30	90				25		
Sphaeralcea coccinea (Pursh) Rydb.					5			
Suaeda calceoliformis (Hook.) Moq.							5	65
Symphoricarpos occidentalis Hook.					5	5		
Taraxacum officinale Weber *	55	10				40	10	
Thermopsis rhombifolia (Nutt.) Richards.					15			
Thlaspi arvense L.*					5			
Tragopogon dubius Scop. *	5			5	10			
Triglochin maritima L.						5	5	

Appendix D1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Achillea millefolium L.				5		
Agropyron pectiniforme R. & S. *	10			15		25
Agropyron smithii Rydb.		5		65	85	75
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	25		85	50		100
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte				5		
Arabis holboellii Hornem.						
Artemisia cana Pursh						
Artemisia frigida Willd.				5	75	
Artemisia ludoviciana Nutt.						
Aster ericoides L.	5	5	10			
Avena fatua L. *						
Axyris amaranthoides L. *						
Bouteloua gracilis (HBK.) Lag.						
Bromus inermis Leyss. *	5			20	80	
Carex spp.						
Carex spp.						
Chenopodium album L. *		5		5		25
Cirsium arvense (L.) Scop. *				30	5	20
Cryptantha fendlerii (A. Gray) Greene				20		40
Descurainia sophia (L.) Webb. *				5	10	
Distichlis stricta (Torr.) Rydb.	85	45	100			
Dracocephalum parviflorum Nutt.						
Elaeagnus commutata Bernh. *ex* Rydb.				5		
Elymus canadensis L.						
Elymus junceus Fisch.						
Erigeron canadensis L.					10	
Erysimum asperum (Nutt.) DC.						

Appendix D1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Euphorbia glyptosperma Engelm.						
Glycyrrhiza lepidota (Nutt.) Pursh						
Grindelia squarrosa (Pursh) Dunal	10	5	35			
Gutierrezia sarothrae (Pursh) Britt. & Rusby						
Helianthus nuttallii T. & G.						
Heterotheca villosa (Pursh) Shinners					55	
Hordeum jubatum L.		90	5			
Juncus longistylis Torr.						
Kochia scoparia (L.) Schrad. *	20	35	15	25		75
Koeleria macrantha (Ledeb.) J.A. Schultes f.						
Lepidium densiflorum Schrad.	5					
Lygodesmia juncea (Pursh) D. Don						
Medicago sativa L. *				10	15	
Melilotus alba Desr. *						
Melilotus officinalis (L.) Lam. *						
Oenothera biennis L.						
Petalostemon purpureum (Vent.) Rydb.						
Plantago eriopoda Torr.						
Poa canbyi (Scribn.) Piper	85	25	10			
Poa pratensis L.				15	50	
Polygonum arenastrum Jord. *ex* Bor.						
Polygonum convolvulus L.						
Puccinellia nuttalliana (Schult.) A.S. Hitchc.			10			
Rosa arkansana Porter				5		
Rumex stenophyllus Ledeb.						
Salicornia europaea L.						
Salsola kali L. *						
Scirpus paludosus A. Nels.						
Setaria glauca (L.) Beauv.						

Appendix D1
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Setaria viridis (L.) Beauv. *						
Solidago canadensis L.						
Solidago graminifolia (L.) Salisb.						
Sonchus arvensis L. *						
Sphaeralcea coccinea (Pursh) Rydb.						
Suaeda calceoliformis (Hook.) Moq.						
Symphoricarpos occidentalis Hook.				5		
Taraxacum officinale Weber *						
Thermopsis rhombifolia (Nutt.) Richards.						
Thlaspi arvense L.*						
Tragopogon dubius Scop. *	15	5				
Triglochin maritima L.						

*exotic

Appendix D2 Alphabetical list of all plant species sampled in 14 uncontaminated plots. All values are frequency (% quadrats with at least one individual)

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Achillea millefolium L.		40		60				
Agropyron pectiniforme R. & S. *	15		10		40			
Agropyron smithii Rydb.	15			45				
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	25	60	5		20	25		25
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte				15		10		
Amelanchier alnifolia Nutt.						5		
Anemone multifida Poir.	5	10						
Antennaria parvifolia Nutt.		10		15				
Arabis holboellii Hornem.								
Artemisia cana Pursh					5			
Artemisia frigida Willd.	70			25	5			
Artemisia ludoviciana Nutt.	5	20		13				
Aster ericoides L.	70	90		45	10		10	
Aster puniceus L.		35						55
Astragalus pectinatus Dougl. *ex* Hook.				5				
Avena fatua L. *					5			
Bouteloua gracilis (HBK.) Lag.	20			50				
Bromus inermis Leyss. *	45		20		30	10	45	
Campanula rotundifolia L.		5						
Carex aquatilis Wahlenb.								75
Chenopodium album L. *							40	
Cirsium arvense (L.) Scop. *		25				70		40
Cryptantha fendlerii (A. Gray) Greene								
Descurainia sophia (L.) Webb. *					5		5	
Distichlis stricta (Torr.) Rydb.								
Elaeagnus commutata Bernh. *ex* Rydb.	40	35		65				
Elymus canadensis L.			10					

Appendix D2
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Erigeron canadensis L.								
Fragaria virginiana Duchesne		5				35		
Glycyrrhiza lepidota (Nutt.) Pursh								
Grindelia squarrosa (Pursh) Dunal					30	30	35	
Gutierrezia sarothrae (Pursh) Britt. & Rusby	25							
Helianthus nuttallii T. & G.								10
Heterotheca villosa (Pursh) Shinners	40				15			
Hordeum jubatum L.			10		30	5	50	15
Iva xanthifolia Nutt.								
Juncus balticus Willd.								15
Kochia scoparia (L.) Schrad. *			5		5		15	
Koeleria macrantha (Ledeb.) J.A. Schultes f.	15							
Lactuca pulchella (Purdh) DC.					10			
Lathyrus venosus Muhl.						10		
Liatris punctata Hook.	5							
Linum lewisii Pursh	5	25						
Medicago sativa L. *					5		45	
Melilotus alba Desr. *	5							
Melilotus officinalis (L.) Lam. *					25	40		
Mentha arvensis L.								5
Muhlenbergia asperifolia (Nees & Mey.) Parodi		20						
Muhlenbergia richardsonis (Trin.) Rydb.		30						
Orthocarpus luteus Nutt.		5		5				
Oxytropis monticola A. Gray	5							
Petalostemon purpureum (Vent.) Rydb.	10	10		10				
Phalaris arundinacea L.								30
Plantago eriopoda Torr.							10	
Poa canbyi (Scribn.) Piper								
Poa pratensis L.	45	55	100	65	65	90		

Appendix D2
continued

Species	Arcola 1	Arcola 2	Cantuar	Forget	Fosterton	Hassard	Manor 1	Manor 2
Populus tremuloides Michx.				5		25		
Potentilla concinna Richards.								
Potentilla pensylvanica L.	10	5						
Psoralea esculenta Pursh	15			5				
Puccinellia nuttalliana (Schult.) A.S. Hitchc.								
Rosa arkansana Porter	45	35		30				
Rubus idaeus L.						20		
Rumex occidentalis S. Wats								
Salicornia europaea L.							20	
Setaria viridis (L.) Beauv. *					15			
Smilacina stellata (L.) Desf.		5						
Solidago canadensis L.	15	70						
Solidago graminifolia (L.) Salisb.	5							5
Solidago rigida L.	5							
Sonchus arvensis L. *		20					10	40
Spartina pectinata Link		5						40
Stellaria longifolia Muhl.								
Stipa comata Trin. & Rupr.	15			20				
Stipa curtiseta (A.S. Hitchc.) Barkworth	30							
Stipa viridula Trin.	10							
Suaeda calceoliformis (Hook.) Moq.							5	
Symphoricarpos occidentalis Hook.		75		25		60		
Taraxacum officinale Weber *		5		5		40	5	
Thermopsis rhombifolia (Nutt.) Richards.				15				
Tragopogon dubius Scop. *	5					5		
Vicia americana Muhl.		5						

Appendix D2
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Achillea millefolium L.			10	10		15
Agropyron pectiniforme R. & S. *		15				
Agropyron smithii Rydb.		10		70	90	65
Agropyron trachycaulum (Link) Malte var. *trachycaulum*	25	90	55			
Agropyron trachycaulum (Link) Malte var. *unilaterale* (Cassidy) Malte						
Amelanchier alnifolia Nutt.						
Anemone multifida Poir.						
Antennaria parvifolia Nutt.				10		25
Arabis holboellii Hornem.				5		
Artemisia cana Pursh						
Artemisia frigida Willd.		5			80	
Artemisia ludoviciana Nutt.						
Aster ericoides L.		10	40			
Aster puniceus L.						
Astragalus pectinatus Dougl. *ex* Hook.						
Avena fatua L. *						
Bouteloua gracilis (HBK.) Lag.						
Bromus inermis Leyss. *		10		30	70	55
Campanula rotundifolia L.						
Carex aquatilis Wahlenb.						
Chenopodium album L. *	5					
Cirsium arvense (L.) Scop. *				25		25
Cryptantha fendlerii (A. Gray) Greene				10		
Descurainia sophia (L.) Webb. *			10	5	5	
Distichlis stricta (Torr.) Rydb.	85		35			
Elaeagnus commutata Bernh. *ex* Rydb.						10
Elymus canadensis L.						

Appendix D2
continued

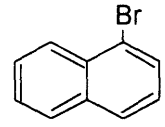
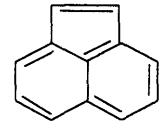
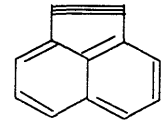
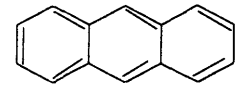
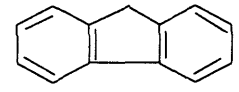
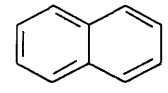
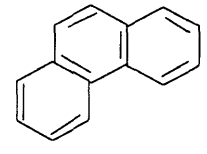
Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Erigeron canadensis L.					5	
Fragaria virginiana Duchesne						
Glycyrrhiza lepidota (Nutt.) Pursh						
Grindelia squarrosa (Pursh) Dunal	5	10	25			
Gutierrezia sarothrae (Pursh) Britt. & Rusby						
Helianthus nuttallii T. & G.						
Heterotheca villosa (Pursh) Shinners	5				55	5
Hordeum jubatum L.	50	30	55			
Iva xanthifolia Nutt.	25					
Juncus balticus Willd.						
Kochia scoparia (L.) Schrad. *		20				
Koeleria macrantha (Ledeb.) J.A. Schultes f.						
Lactuca pulchella (Purdh) DC.						
Lathyrus venosus Muhl.						
Liatris punctata Hook.						
Linum lewisii Pursh						
Medicago sativa L. *				5	25	40
Melilotus alba Desr. *						
Melilotus officinalis (L.) Lam. *			5			
Mentha arvensis L.						
Muhlenbergia asperifolia (Nees & Mey.) Parodi						
Muhlenbergia richardsonis (Trin.) Rydb.						
Orthocarpus luteus Nutt.						
Oxytropis monticola A. Gray						
Petalostemon purpureum (Vent.) Rydb.						
Phalaris arundinacea L.						
Plantago eriopoda Torr.						
Poa canbyi (Scribn.) Piper	30	15	100			
Poa pratensis L.				40	70	75

Appendix D2
continued

Species	Success 1	Success 2	Success 3	Winter 1	Winter 2	Winter 3
Populus tremuloides Michx.						
Potentilla concinna Richards.				5		
Potentilla pensylvanica L.				10		30
Psoralea esculenta Pursh						
Puccinellia nuttalliana (Schult.) A.S. Hitchc.	70		20			
Rosa arkansana Porter				25		15
Rubus idaeus L.						
Rumex occidentalis S. Wats			5			
Salicornia europaea L.						
Setaria viridis (L.) Beauv. *						
Solidago canadensis L.						5
Solidago graminifolia (L.) Salisb.				5		
Solidago rigida L.						
Sonchus arvensis L. *	5		10			
Spartina pectinata Link						
Stellaria longifolia Muhl.					5	5
Stipa comata Trin. & Rupr.						
Stipa curtiseta (A.S. Hitchc.) Barkworth						
Stipa viridula Trin.						
Suaeda calceoliformis (Hook.) Moq.	15					
Symphoricarpos occidentalis Hook.				55		30
Taraxacum officinale Weber *						
Thermopsis rhombifolia (Nutt.) Richards.						
Tragopogon dubius Scop. *			5			
Vicia americana Muhl.				5		

* exotic

Appendix E Chemical structures of polycyclic aromatic hydrocarbons.

Formula	Hydrocarbon	Chemical Structure
$C_{10}H_7Br$	2-bromonaphthalene	
$C_{12}H_8$	Acenapthene	
$C_{12}H_6$	Acenaphthylene	
$C_{14}H_{10}$	Anthracene	
$C_{13}H_9$	Fluorene	
$C_{10}H_8$	Naphthalene	
$C_{14}H_{10}$	Phenanthrene	
$C_{16}H_{10}$	Pyrene